



GENETICS OF HEREDITARY HEMOCHROMATOSIS

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DEFINITION OF HEMOCHROMATOSIS

- Any disorder that is characterized by tissue injury due to iron overload in many organs



DIAGNOSIS OF IRON OVERLOAD

- Clinical
- Serum iron studies
 - TRS
 - Ferritin
- Measurement of HIC
 - Biochemical measurement
 - MRI
- Quantitative phlebotomy

Ayonrinde et al Crit Rev Clin Lab Sci 2008;45:451
Olynyk et al Hepatology 2008;48:991



KEY IRON OVERLOAD DISORDERS

- **Hereditary Hemochromatosis**
 - **Type 1 (*HFE*-related)**
 - C282Y homozygous
 - C282Y/H63D compound heterozygous
 - Other *HFE* mutations
 - **Type 2 (juvenile hemochromatosis)**
 - A. hemojuvelin (*HJV*) mutations
 - B. hepcidin (*HAMP*) mutations
 - **Type 3 (*TFR2* mutations)**
 - **Type 4 (ferroportin mutations)**
 - A. loss-of-function
 - B. gain-of-function
- **Secondary Iron Overload**
 - Iron-loading anemias
 - Parenteral iron overload
 - Long-term hemodialysis
 - Chronic liver disease
 - Alcoholic liver disease
 - Hepatitis B or C
 - Porphyria cutanea tarda
 - Nonalcoholic steatohepatitis
 - **Miscellaneous**
 - Congenital alloimmune hepatitis (neonatal iron overload)
 - African iron overload
 - Aceruloplasminemia
 - Atransferrinemia

Olynyk et al Hepatology 2008;48:991-1001

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 - A. loss-of-function
 - B. gain-of-function

In all types of HH (except 4A)

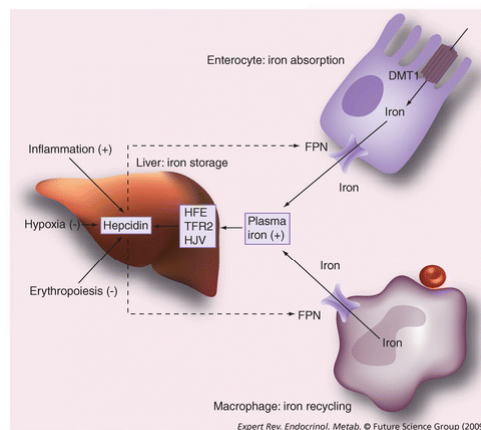
↓
reduced hepcidin (*HAMP*)
expression or activity

↓
impaired ferroportin (*FPN*)
degradation

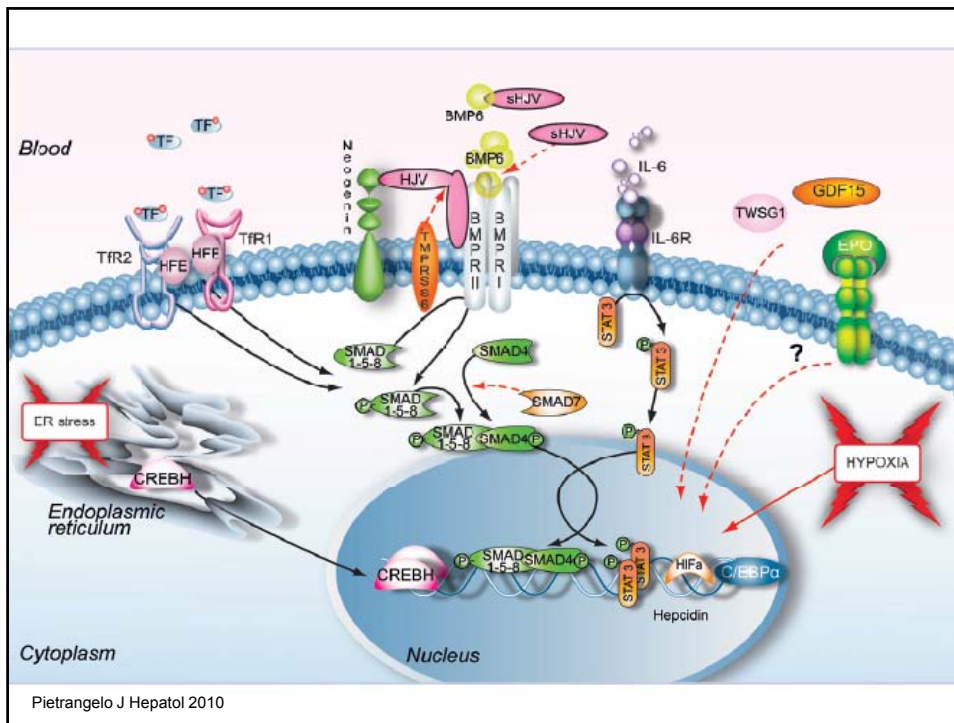
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increased iron export from
enterocytes and macrophages

Olynyk et al Hepatology 2008;48:991-1001

HEREDITARY HEMOCHROMATOSIS: AN ENDOCRINE DISORDER



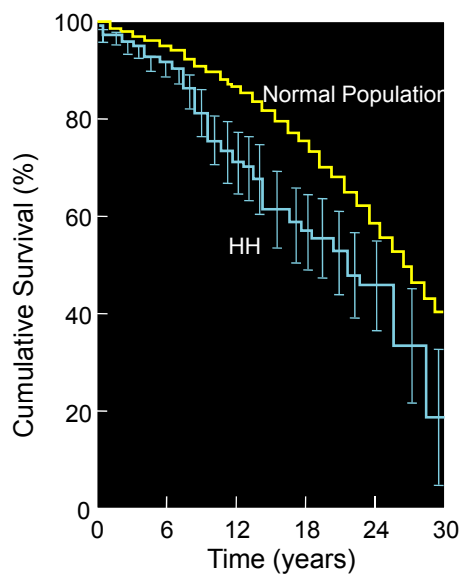
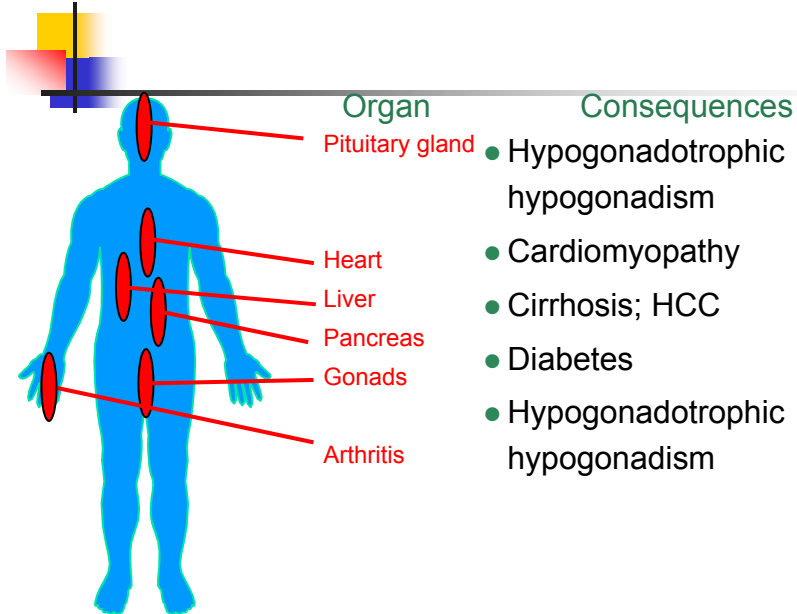
Gan et al Expert Rev Endocrinol Metab 2009;4:229



CLINICAL ASPECTS OF HEREDITARY HEMOCHROMATOSIS

THE PAST

CLINICAL CONSEQUENCES



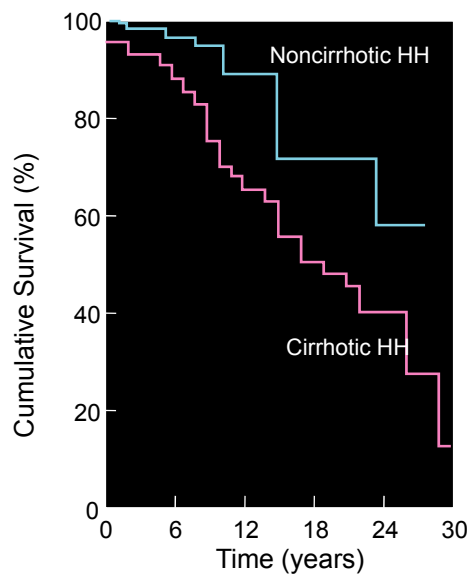
Niederer et al. 1996

Death Rates in 251 Patients with HH Compared with Expected Rates in Normal Population

Cause of Death (n = 69)	% of Deaths	Mortality Ratio Observed / Expected
Liver Cancer	28	119
Diabetes Mellitus	5	14
Cardiomyopathy	7	14
Cirrhosis	20	10
Total	60	

Causes of death in remaining 40% were no more frequent than expected in matched population.

Niederer et al. 1996



Niederer et al. 1996



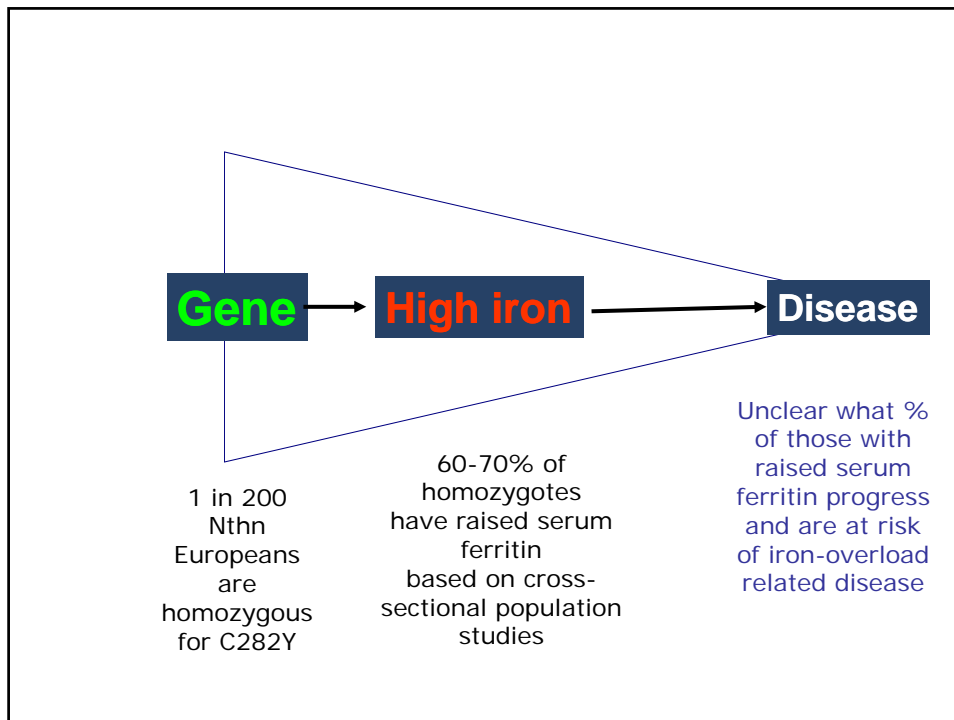
CLINICAL ASPECTS OF HEREDITARY HEMOCHROMATOSIS

THE PRESENT



MORTALITY DUE TO HEREDITARY HEMOCHROMATOSIS

- **No increased risk of death compared to wild-type subjects at population level**
 - Van Aken et al Eur J Clin Invest 2002; Willis et al Blood Cells Mol Dis 2003; Waalen et al Best Pract Res Clin Hematol 2005; Allen et al NEJM 2008; Busse et al Population Study
- **Cirrhotics have increased mortality and morbidity**
 - Niederau et al NEJM 1985; Niederau et al Gastroenterol 1996; Wojcik et al Can J Gastroenterol 2002



MUTATIONS IN THE *HFE* GENE ARE COMMON

- 1:7 individuals is heterozygous for C282Y
- 1:3 individuals is heterozygous for H63D
- 1:42 compound heterozygote C282Y/H63D
- 1:190 individuals is homozygous for C282Y



ASSUMPTION

- Serum ferritin levels reflect iron stores
 - Ferritin < 20 µg/L = Fe depletion ✓
 - Ferritin < 10 µg/L = Fe deficiency ✓
 - What about ferritin > ULN?

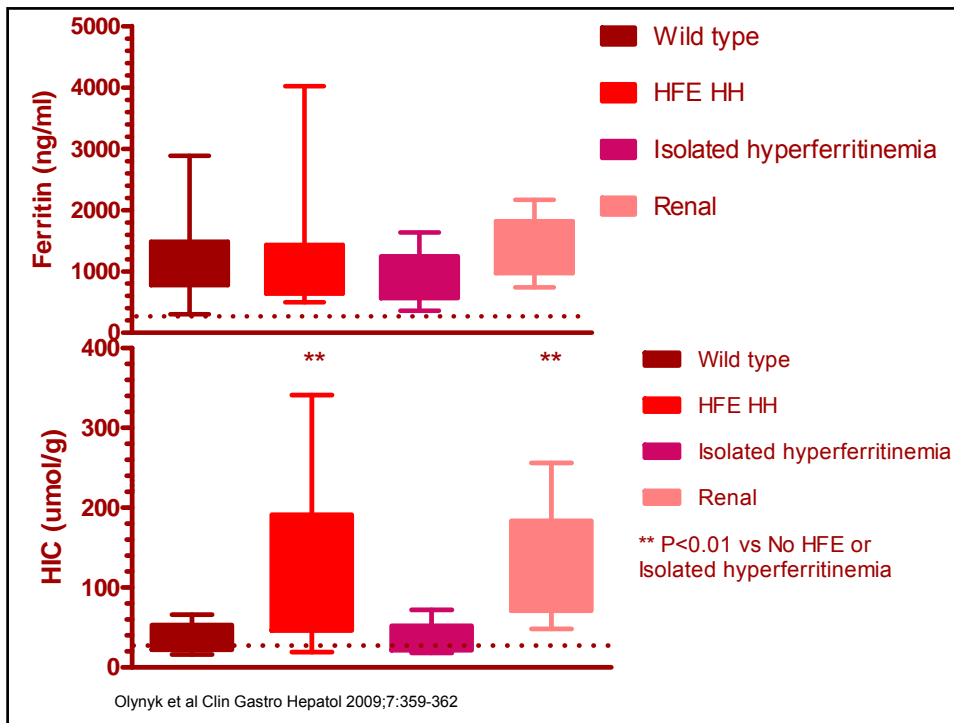
Clark Curr Opinion Gastroenterol 2009



ELEVATIONS OF SERUM IRON STUDIES ARE COMMON

- 13% Australian population have elevated serum ferritin levels
- 6% Australian population have elevated transferrin saturation
- 40% of haemodialysis patients have ferritin levels > 500 µg/L
- 20% of adults have fatty liver
 - 30-50% of these can have elevated ferritin levels

Olynyk et al NEJM 1999; Ombiga et al Semin Liv Dis 2005; ANZDATA 2009;5:19

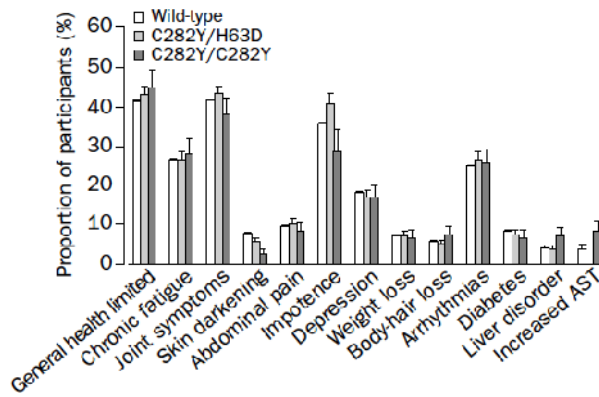


CROSS SECTIONAL POPULATION STUDIES

	Sample	C282Y homozygosity	% elevated ferritin	Disease	Fibrosis Cirrhosis
Olynyk 1999	3011	1:190	50%	P,A,L	3/11 (27%) 1/11 (9%)
Asberg 2001	65238	Unknown	Unknown	ALT,F,A	13/153 (8%) 4/148 (3%)
Beutler 2002	41038	1:270	76% men 54% women	ALT	??25%
Deugnier 2002	9396	1:175	70% men 33% women	F,A	Unknown
HEIRS 2005	99711	1:227	88% men 57% women	Liver disease (men only)	Unknown
Delatycki 2005	11307	1:240	Unknown	L	4/4 ?



THE CONTROVERSY



Beutler et al Lancet 2002



LONGITUDINAL POPULATION STUDIES

	Sample C282Y homozygotes	% ferritin elevated at baseline	Duration of follow-up	Biochemical progression	Fibrosis/ Cirrhosis at end of study
Olynyk 2004	10	40%	17 years	40%	30%
Andersen 2004	23	80% men 30% women	25 years	20%	Unknown
Allen 2008	203	84% men 65% women	12 years		

Table 5. Summary of Overall Evidence

Key Question	Studies, n	Study Designs (Reference)	Quality	Conclusions
1. Penetrance of hemochromatosis	11	1 retrospective cohort study (46)	Good: Genotyping of surviving Brusselton, Australia, cohort; potential selective mortality bias appears minimal. Small numbers.	17 y of clinical data for 10 screening-detected general population C282Y homozygotes illustrates variable disease expression and incomplete penetrance. Incomplete follow-up into older age where disease penetrance increases.
		1 retrospective and prospective cohort study (47)	Fair: Genotyping of representative Danish cohort during third examination. Results are likely to be compromised by selective mortality bias due to 35% loss of follow-up. Even accounting for potential bias, disease penetrance about 60%.	Additional 23 screening-detected C282Y homozygotes from the general population also illustrates variable disease penetrance and variable patterns of iron accumulation. No liver biopsies to confirm iron overload or disease.
		9 cross-sectional studies (32, 51-58)	Fair to good: Studies compromised by frequent inclusion of already-identified C282Y homozygotes (not clearly screening-detected), by different standards for disease, and by potential selection bias due to non-protocol-based selection for further clinical work-up.	Estimates of disease in newly identified C282Y homozygotes at screening are too limited to provide confident estimates of penetrance.
2. Efficacy of phlebotomy treatment	5	4 case series (25, 58-60)	Fair to poor: Studies compromised by selective samples, reporting on cases not clearly comparable to current diagnosis and treatment, incomplete follow-up on all cases, and failure to account for possible confounders in analyses.	Total number of reported cases is quite small and represents disease experience over 50 y. There are no data to determine the benefit of earlier treatment among screening-detected compared with contemporarily diagnosed clinical cases.
		1 retrospective survey (55)	Fair: Possible recall bias in determining response to treatment.	Treatment is recalled to relieve some but not all symptoms in a survey of patients with hereditary hemochromatosis.
3. High-risk groups	7	7 cross-sectional studies (51, 57, 61-63, 65, 66)	Fair to good: Studies examined prevalence of C282Y homozygotes in various selective populations for possible targeted screening.	Patients selected on basis of certain signs and symptoms, in combination with phenotypic testing, may be at increased risk; data are still fairly limited.

Whitlock, E. P. et. al. Ann Intern Med 2006;145:209-223

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HealthIron: Sub-study of the Melbourne Collaborative Cohort Study

1990-1994

MCCS baseline

(mean age 54 yrs at baseline)

Questionnaire
Physical Examination
Blood sample

2003-2006

MCCS follow-up

(mean age 66 yrs at baseline)

Questionnaire
Physical Examination
Blood sample

HealthIron recruitment

Genotyping of HFE mutations from 31,192 baseline samples

WHICH SUBJECTS WITH UNTREATED HH PROGRESS?

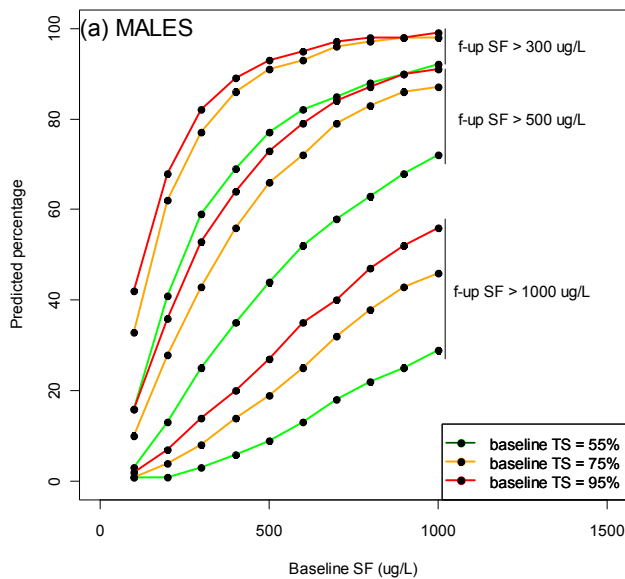
- 31,192 subjects in MCCS
 - 203 HFE HH (mean age 55 years) with baseline iron studies
 - 86 were previously undiagnosed and had baseline iron studies and studies at follow-up 12 years later

WHICH SUBJECTS WITH UNTREATED HH PROGRESS?

■ At baseline

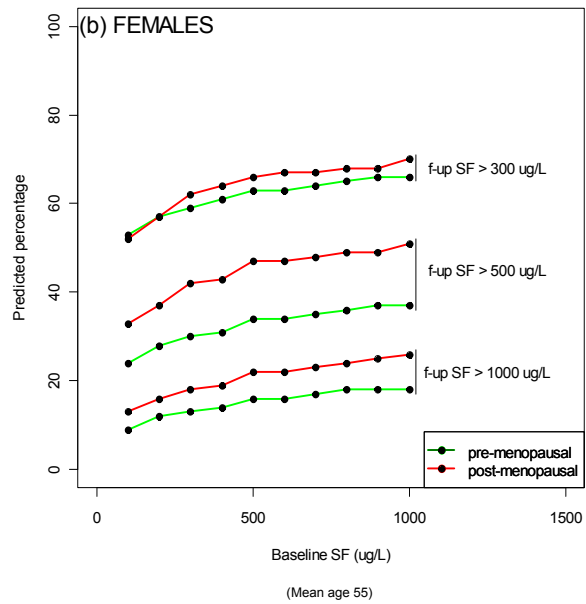
- 84% male and 65% female HH subjects had ferritin > ULN
- 37% male and 3% female HH had ferritin > 1000 ug/L

Allen et al New Engl J Med 2008;358:221-30
Gurrin et al Gastroenterology 2008;135:1945-52



Gurrin et al Gastroenterology 2008;135:1945-52

(Mean age 55)



Gurrin et al Gastroenterology 2008;135:1945-52

WHO DEVELOPS IRON OVERLOAD-RELATED DISEASE?

Evidence of iron overload

(HIC > 90 $\mu\text{mol/g}$, ferritin > 1000 $\mu\text{g/L}$ and phlebotomy therapy)

AND

features of HH-associated disease

(HCC, liver disease, cirrhosis, arthritis or clinical problem with no other explanation)

Allen et al New Engl J Med 2008;358:221-30



IRON OVERLOAD-RELATED DISEASE

- At age 65 years
 - 28% of men
 - 1% of women
- Arthritis
 - Bilateral MCPJ, hip or large joint arthropathy in 24% of HH subjects with ferritin > 1000

Allen et al New Engl J Med 2008;358:221-30
Carroll et al Arthritis Rheum 2010 October 15 (epub ahead of print)



WHAT ABOUT COMPOUND HETEROZYGOTES?

- 180 C282Y/H63D (84 male) and 330 randomly selected wild-type subjects
- Followed for 12 years (age 53 to 65)
- Higher mean ferritin and TRS values compared with wild-type (but still normal). No increase over 12 years.
- 1 male and 0 females iron overload disease

Gurrin et al Hepatology 2009;50:94-101



WHAT ABOUT C282Y HOMOZYGOTES WITH FERRITIN < 1000 ?

- 90 C282Y homozygotes (29 male, 61 female) and 329 randomly selected wild-type subjects with ferritin at baseline between ULN and 1000 µg/L
- Followed for 12 years (age 55 to 67)
- Only 1 male increased to ferritin > 1000 µg/L over 12 years.
- No increased risk of HH clinical features or iron overload disease compared with wild type controls OR C282Y homozygotes with normal ferritin levels
- Treatment may not be necessary

Allen et al Hepatology 2010;52:925-33



HFE AND CANCER

- C282Y homozygosity
 - 3-fold increased risk of colorectal cancer in men and women
 - 3-fold increased risk of breast cancer in women
- H63D homozygosity
 - Genetic modifier of cancer development in HNPCC with mutations in MMR genes
 - 3-fold increased risk of cancer in MMR gene mutation carriers and earlier onset of cancer

Osborne et al. Hepatology 2010;51:1311-18
Shi et al. Int J Cancer 2009;125:78-83

GENETIC AND ENVIRONMENTAL MODIFIERS

	GENETIC	ENVIRONMENTAL
Iron loading	Modifying genes <ul style="list-style-type: none"> • <i>CYBRD1</i> • <i>HAMP</i> • <i>HJV</i> • <i>BMP</i> • <i>TMPRSS6</i> • <i>Transferrin</i> • <i>Hp</i> • <i>Beta thalassemia trait</i> 	Alcohol (>60g/day) <ul style="list-style-type: none"> • Increased iron loading possibly secondary to <i>de novo</i> ferritin synthesis Diet <ul style="list-style-type: none"> • Data currently inadequate • Heme iron intake- positive correlation much stronger in post-menopausal women] Non-citrus fruit <ul style="list-style-type: none"> • Confers protection
Fibrogenesis	Gender <ul style="list-style-type: none"> • Male- more rapid progression • Female- estrogen; possible antioxidant effect Profibrogenic genes <ul style="list-style-type: none"> • <i>TGFβ</i>- cirrhosis at younger age • <i>TNFα, collagen IV</i>- no clear relationship Proinflammatory genes <ul style="list-style-type: none"> • Monocyte chemotactic protein- in setting of chronic Hepatitis C] Pro-oxidative gene <ul style="list-style-type: none"> • Glutathione S-transferase P1 enzyme- less active Val/val genotype • Myeloperoxidase- greater activity in cirrhotics] • MnSOD polymorphisms- increased cardiomyopathy 	Alcohol <ul style="list-style-type: none"> • Most significant factor • Related to oxidative stress Viral Hepatitis <ul style="list-style-type: none"> • Younger age of cirrhosis presentation, synergistic with HH] Hepatic steatosis <ul style="list-style-type: none"> • Positive correlation with cirrhosis

Gan et al Expert Rev Endocrinol Metab 2009;4:225-239

CYBRD1: A NEW POTENT GENETIC MODIFIER

- 863 subjects from HealthIron study – 121 C282Y homozygotes and 762 non-homozygotes
- 384 SNPs on Illumina Golden Gate and Sequenom iPLEX
- Gene encoding DCytB (duodenal reductase required for non-heme iron absorption)
- rs88409 SNP
 - Minor allele freq 0.165 (c.w. C282Y allelic freq 0.076)
 - 30% reduction in basal promoter activity
- Effect on C282Y homozygotes
 - Wild type – mean ferritin 1047 µg/L
 - 67% ferritin > 1000 µg/L
 - rs88409 1 or 2 alleles – mean ferritin 314 µg/L
 - 21% ferritin > 1000 µg/L
- More potent effect on iron status than recently reported *TMPRSS6* polymorphism
- Accounts for 11% of variance in serum ferritin levels in C282Y homozygotes

Allen et al New Engl J Med 2008;358:221-30
Constantine et al Br J Haematol 2009;147:140-49

HEPCIDIN: AN ESSENTIAL LABORATORY ASSAY

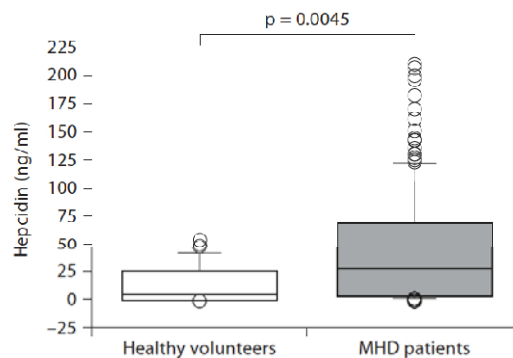
Table 1 Baseline characteristics, iron indicators and inflammation indicators of all the groups

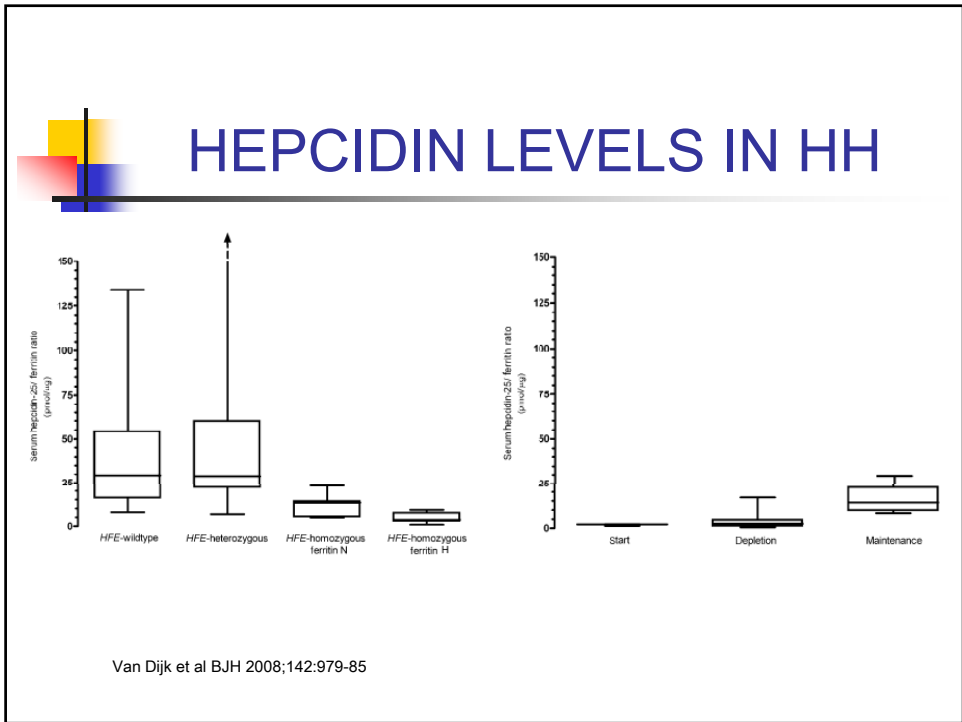
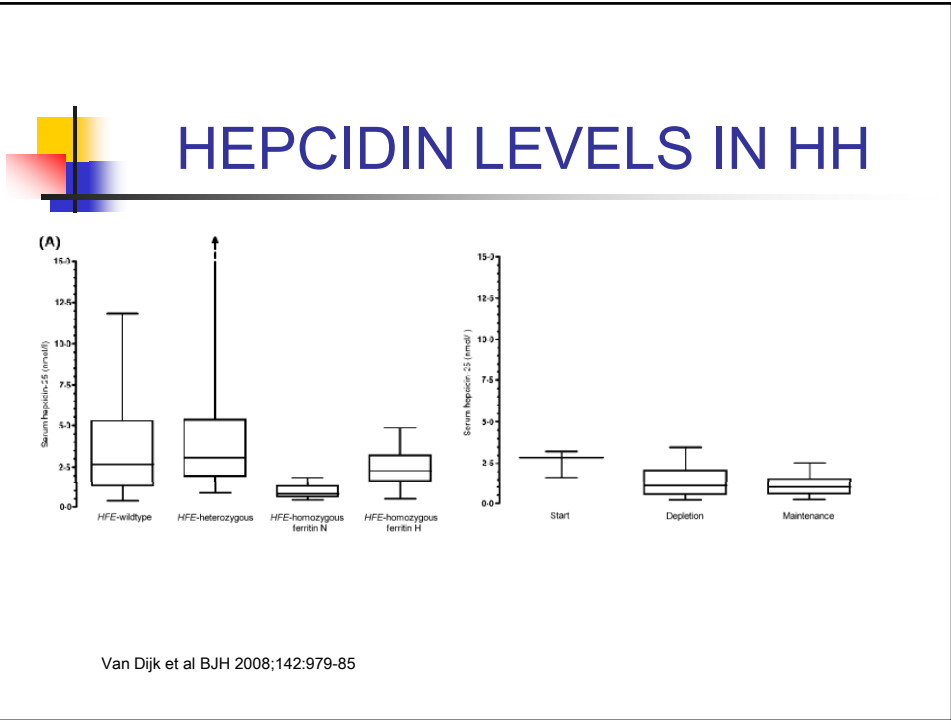
	ACD	ACD/IDA	AcI	IDA	Control
No. patients	16	7	25	16	23
Gender (M/F)	5/11	2/5	14/11	4/12	10/13
Age	54.40 ± 23.02	48.42 ± 24.38	41.04 ± 20.63	35.64 ± 13.32	36.70 ± 14.17
RBC (×10 ¹² /L)	3.18 ± 0.74**	3.55 ± 0.54**	4.02 ± 0.86***	3.82 ± 0.80***	4.65 ± 0.47
Hb	96.27 ± 20.25**	85 ± 23.12**†	112.57 ± 23.47§	74.73 ± 17.53 ***§§††	129.38 ± 14.59
MCH (pg)	30.66 ± 3.53	24.11 ± 5.66***§§†	28.67 ± 3.10	19.65 ± 2.64***§§††	30.63 ± 3.47
MCV (fL)	90.00 ± 9.08	68.22 ± 7.03***§§††	84.75 ± 8.08*	66.23 ± 7.79***§§††	91.11 ± 4.50
Ret (%)	2.00 ± 1.7*	2.49 ± 0.48**††	1.60 ± 0.69*	1.96 ± 0.87***	0.94 ± 0.55
WBC (×10 ⁹ /L)	7.62 ± 2.91††	8.04 ± 2.36††	13.39 ± 5.09**	5.79 ± 1.71††	6.27 ± 1.08
Plt	137.08 ± 83.87**	352.5 ± 166.03***§§	318.70 ± 152.28***§§	31.60 ± 76.40***§§	228.36 ± 44.85
EPO (IU/L)	7.54 ± 6.71*†	8.15 ± 8.01*†	3.30 ± 2.10	8.47 ± 7.05**††	2.77 ± 1.33
Ferritin (µg/L)	612.05 ± 502.68**	7.94 ± 9.58***§§††	640.90 ± 436.99**	3.01 ± 2.03***§§††	133.94 ± 70.44
IL-6 (µg/L)	59.63 ± 73.08**	42.75 ± 87.21**††	142.99 ± 72.32***§§	3.16 ± 4.44§§††	3.00 ± 1.40
CRP (mg/L)	11.8 ± 10.51†	9.13 ± 3.09**†	45.41 ± 30.32**	2.61 ± 2.46§††	2.27 ± 1.35
Hepcidin (µg/L)	434.83 ± 217.00**	238.32 ± 93.85§†	410.08 ± 299.96**	110.79 ± 19.22***§§††	177.58 ± 119.84

M, male, F, female, ACD anemia of chronic diseases, ACD/IDA anemia of chronic disease and concomitant iron-deficiency anemia, AcI acute inflammation, IDA iron-deficiency anemia, WBC white blood cell, RBC red blood cell, MCV mean cell volume, Plt platelet, Hb hemoglobin, MCH: mean corpuscular hemoglobin, Ret reticulocyte, EPO erythropoietin, IL-6 interleukin 6, CRP C-reative protein

Cheng Clin Exp Med DOI 10.1007/s10238-010-0102-9

HEPCIDIN IN CHRONIC KIDNEY DISEASE







TAKE HOME MESSAGE

- Men and women > 55 years with normal ferritin and TRS – almost no chance of progression to iron overload disease
- Cancer screening advice to both genders
- Need access to serum hepcidin!



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