The 1-1 Haptoglobin Phenotype is Associated With Perihematoma Edema Progression in Acute intracranial Hemorrhage

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Presented:

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Background

- Case-Fatality rate of **25-30%** in high income countries, **30-48%** in low/middle income countries
- **20%** have good functional outcome at 6 months

- Trajectory of Perihematomal Edema (PHE) in ICH predicts 90 day mortality and functional outcomes
- PHE develops due to Mechanical forces (primary injury) but also **Inflammation** (secondary injury)
  - RBC lysis -> ROS -> MMP-9,TNF-α,IL-10,NF-κB
  - BBB breakdown-> PHE formation

1. Koton S, Schneider AL, Rosamond WD, et. al. Stroke Incidence and mortality trends in US communities, 1987 to 2011. JAMA. 2014; 312 (3): 259-268
2. Feigin VL, Lawes CMM, Bennett DA et. al. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. Lancet Neurol. 2009; 355-369
3. van Asch CJJ, Luitse MJA, Rinkel GJE et. al. Incidence, case fatality, and functional outcome of intracerebral hemorrhage over time, according to age, sex, and ethnic origin: a systematic review and meta-analysis. Lancet Neurol 2010; 9: 167-76

Haptoglobin and Neuroinflammation

- Haptoglobin (Hp): an acute-phase reactant, 2 α and 2 β chains,
  - Three main Human phenotypes Hp 1-1, Hp 2-1, Hp 2-2
  - binds extracellular hemoglobin after RBC lysis to **prevent ROS formation** and **increase clearance**
- Studies indicate Hp1-1 and 2-2 bind Hb with similar affinity.
  - Macrophage CD163 receptor has an approximately **eight times greater affinity for Hp2-2** than Hp1-1
  - More **rapid uptake of the Hp(1-1)–Hb complex**
- **Therefore** Hp1-1 may be more effective in preventing oxidative damage
  - Hp has previously been associated with functional outcomes in ICH and aSAH, as well as Vasospasm in aSAH.

- **We predict Hp 1-1 to have decreased PHE formation over the first 96 hours compared to the Hp 2-1 or 2-2 genotypes.**

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8. Aronowski J, Zhao X. Molecular pathophysiology of cerebral hemorrhage: secondary brain injury. Stroke. 2011; 42: 1781-1786.

Methods

- **87 Prospectively** collected subjects 18 years or older from 2 tertiary care centers
  - The Johns Hopkins Hospital
  - The Massachusetts General Hospital
- **Baseline admission CTH and follow-up within 96 hours**
  - PHE was completed by semi-automated (OsiriX) approach by blinded reviewers
- Blood samples collected prior to product administration, gel electrophoresis for Haptoglobin Genotype
- **Primary outcome: PHE progression**
  - Secondary Outcome: ICH volume, mortality, DC functional recovery
- Statistical analysis using SAS (Cary, NC)
  - Baseline characteristics were compared using appropriate statistical tests
    - Pearson chi^2 and fisher for categorical variables, one way ANOVA for normally distributed continuous variables and kruskal Wallis testing for non-normally distributed variables.
  - Univariate logistic regression was used to analyze the relationship between Haptoglobin genotype and outcomes of interest
  - Results were considered significant when a $p < 0.05$ was reached.

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Baseline Characteristics

Pearson chi^2 or fisher exact test for categorical variables, one way ANOVA for normally distributed continuous variables and kruskal wallis testing for non-normally distributed variables.

	Hopkins	MGH	Total	
N	57	30	87	
Age (mean, SD)	59.8 (16)	65 (12)	61.7 (15)	0.1162
Male (N, %)	32 (56)	11 (37)	43 (49)	0.0842
African American (N, %)	26 (46)	2 (7)	28 (33)	**<0.0001**
HTN (N, %)	43 (75)	21 (70)	64 (74)	0.5845
HLD (N, %)	5 (9)	12 (40)	17 (20)	**0.0005**
Smoking (N, %)	20 (35)	2 (7)	22 (25)	**<0.0001**
Current Medication (N,%)	13 (23)	16 (53)	29 (33)	**0.0041**
Previous ICH (N, %)	5 (9)	4 (13)	9 (11)	0.2163
Haptoglobin (N, %)				
Hp 1-1	6 (11)	3 (10)	9 (10)	**0.0437**
Hp 2-1	28 (49)	17 (57)	45 (51)	
Hp 2-2	23 (40)	10 (33)	33 (38)	
Death	13 (23)	6 (20)	19 (22)	0.76
Poor MRS	47 (82)	16 (53)	63 (72)	0.004

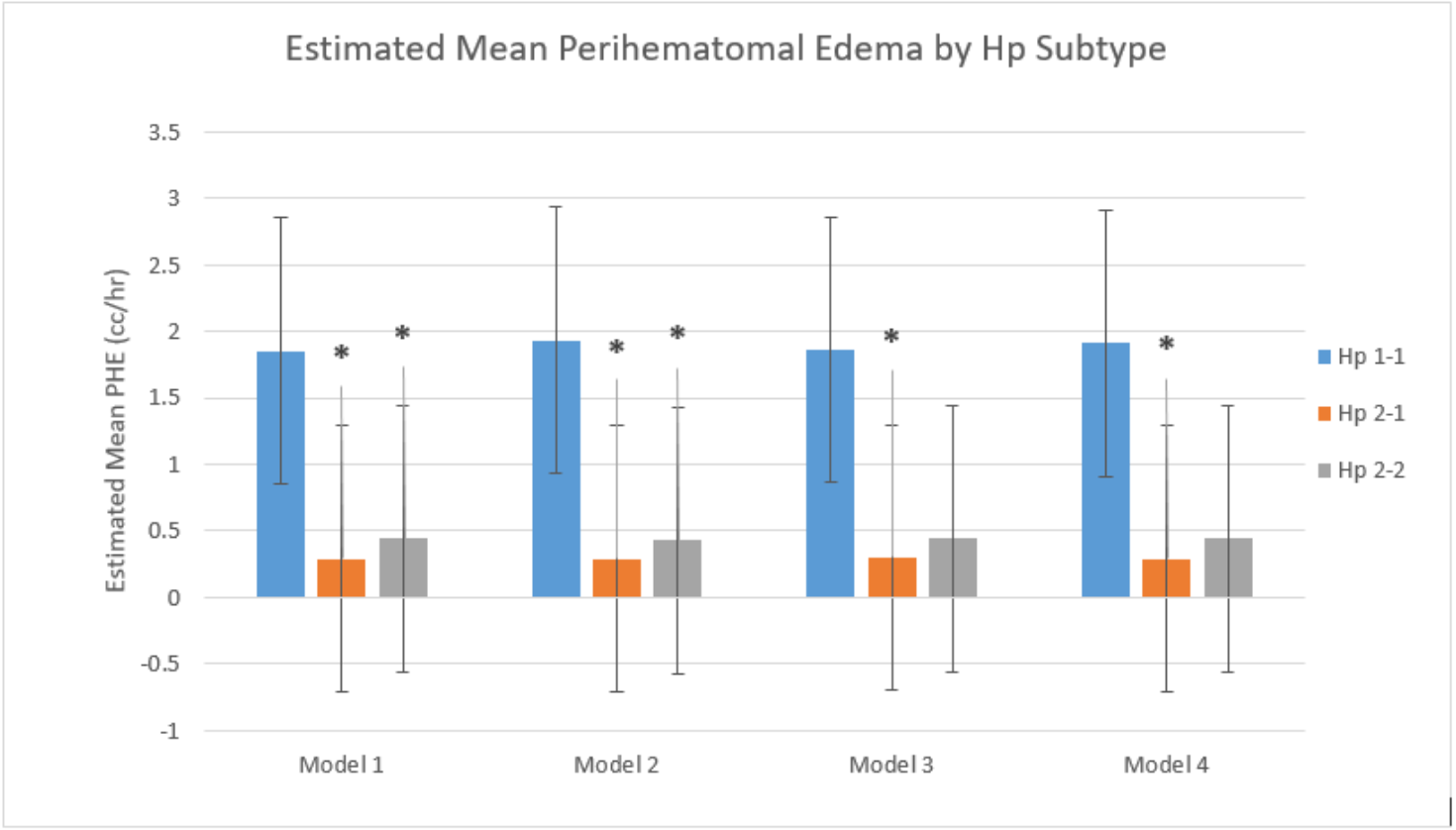

Table 1: Plotted mean PHE by Hp subtype. Brackets represent upper and lower limit 95% confidence interval. Model 1: unadjusted; Model 2: model 1 + age, sex, race; Model 3: model 2 + HTN, HLD, smoking, DM, prior ICH; Model 4: Model 3 + anticoagulation or antiplatelet use at time of presentation. * $p \leq 0.05$, ** $p \leq 0.01$.

	Model 1		Model 2		Model 3		Model 4	
	Estimated Mean (95% CI)	P Value	Estimated Mean (95% CI)	P value	Estimated Mean (95% CI)	P value	Estimated Mean (95% CI)	P value
Hp 1-1	1.85 (0.86, 2.82)	Ref	1.93 (-0.89, 2.96)	Ref	1.86 (0.78, 2.94)	Ref	1.91 (0.79, 3.02)	Ref
Hp 2-1	0.29 (-0.15, 0.72)	0.0146	0.29 (-0.16, 0.74)	0.0152	0.30 (-0.17, 0.76)	0.032	0.29 (-0.19,0.77)	0.0309
Hp 2-2	0.44 (-0.06, 0.95)	0.0407	0.43 (-0.09, 0.95)	0.038	0.44 (-0.10, 0.98)	0.068	0.44 (-0.11, 0.99)	0.0694

Table 2: Adjusted mean PHE by model; Model 1: unadjusted; Model 2: model 1 + age, sex, race; Model 3: model 2 + HTN, HLD, smoking, DM, prior ICH; Model 4: Model 3 + anticoagulation or antiplatelet use at time of presentation

Secondary Outcomes of Interest by Hp Genotype

Odds of mortality and Poor discharge mRS (>4) by Hp Genotype.

Unadjusted Odds ratio

* p<0.05, ** p<0.001

	Genotype	OR (95% CI)
Mortality	Hp 1-1	Reference
	HP 2-1	1.00 (0.179, 5.59)
	HP 2-2	0.94 (0.159, 5.58)
Poor mRS	Hp 1-1	Reference
	HP 2-1	1.0 (0.22, 4.6)
	HP 2-2	2.25 (0.43, 11.6)

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Summary of Results

- Primary Outcome:
  - We observed different rates of PHE within the first 96 hours of ICH by Hp Genotype
    - Hp 1-1 formation rates where more than double Hp 2-1 and 2-2 (1.91cc/hr v 0.29 cc/hr, 0.44 cc/hr)
- Secondary Outcome(s):
  - No difference in Mortality by Hp Genotype
  - Non-significant trend toward poor functional outcome amongst Hp 2-1, 2-2

Is this Biologically Plausible?

- Hp 1-1 **rapid uptake and clearance**-> driving early PHE and earlier peak edema curve
- Neuroinflammation ≠ PHE
  - Is PHE driven more by vasogenic or endothelial factors released in injury
- What about the Mortality/Outcome?
  - Hp 1-1 decreased long term neuroinflammation -> improved functional outcomes

Summary

- Haptoglobin phenotype influences early PHE formation (within the first 96 hours)
- Hp genetics may explain some heterogeneity of PHE formation within ICH
- Our cohort continues to support the observation that Hp genotype influences functional outcomes in ICH reported previously – mediated via PHE?

Thank you for you time!

- Thank you to our patients and their families
- Special Thanks to Paul Nyquist
- Our co-Authors
  - WA Mould, KN Sheth, J Rosand, R Thompson, A Levy, DF Hanley, JN Goldstein

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1. Koton S, Schneider AL, Rosamond WD, et. al. Stroke Incidence and mortality trends in US communities, 1987 to 2011. JAMA. 2014; 312 (3): 259-268

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5. Venkatasubramanian C, Mlynash M, Finley-Caulfield A et. al. Natural history of perihematomal edema after intracerebral hemorrhage measured by serial magnetic resonance imaging. Stroke. 2011; 42: 73-80.

6. Zheng H, Chen C, Zhang J et.al. Mechanism and therapy of brain edema after intracerebral hemorrhage. Cerebrovasc Dis. 2016; 42: 155-169.

9. International Journal of Laboratory Hematology; Volume 29, Issue 2, pages 92-110, 6 MAR 2007 DOI: 10.1111/j.1751-553X.2007.00898.x; http://onlinelibrary.wiley.com/doi/10.1111/j.1751-553X.2007.00898.x/full#f2

10. Murthy SB, Levy AP, Duckworth J et. al. Presence of haptoglobin -2 allele is associated with worse functional outcomes after spontaneous intracerebral hemorrhage. World Neurosurg. 2015; 83, 4: 583-587.

11. Kantor E, Bayir H, Ren D, et. al. Haptoglobin genotype and functional outcome after aneurysmal subarachnoid hemorrhage. J Neurosurg. 2014; 120: 386-390

12. Chaichana KL, Levy AP, Miller-Lotan R et. al. Haptoglobin 2-2 genotype determines chronic vasospasm after experimental subarachnoid hemorrhage. Stroke. 2007; 38: 3266-3271.

13. Lederc JL, Blackburn S, Neal D et. al.; Haptoglobin phenotype predicts the development of focal and global cerebral vasospasm and may influence outcomes after aneurysmal subarachnoid hemorrhage. PNAS. 2015; 112: 4: 1155-1160

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Thank you for you time!

Questions

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Mechanism of injury in ICH

- Bimodal
  - Primary Injury: Hematoma expansion, Minutes to hours, mechanical damage due to mass effect
  - Secondary injury: >6 hours, inflammatory and neuronal toxicity, driving perihematomal edema (PHE)
    - PHE increases most rapidly with 48 hours
    - PHE continues up to 12 days post ICH
- The majority of our current interventions target Primary Injury

4. Kim H, Edwards NJ, Choi HA et. al. Treatement strategies to attenuate perihematomal edema in pateints with intracerebral hemorrhage. World Neurosurg. 2016. 94: 32-41.
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Conceptual model of the stages of Neuroinflammation

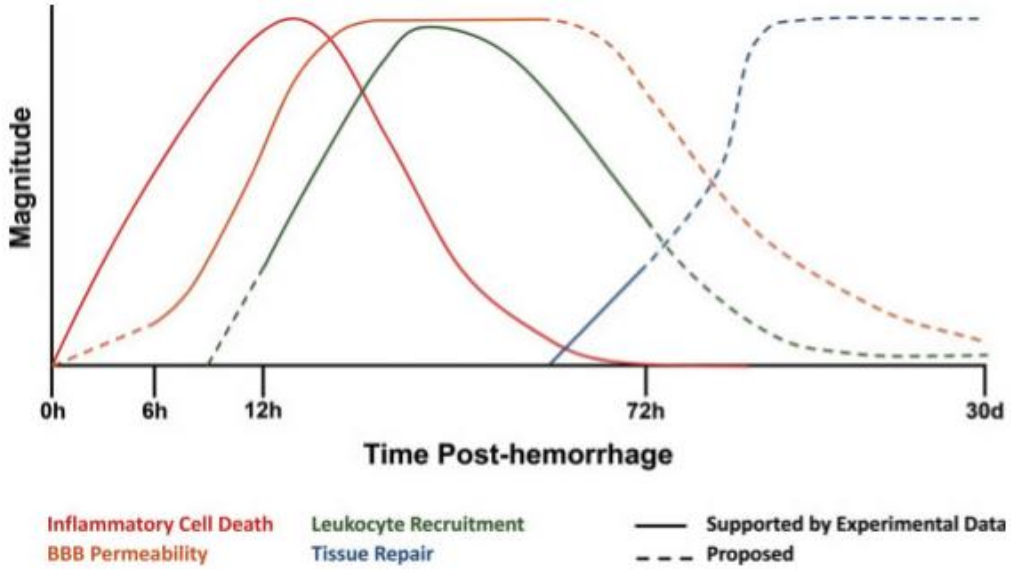

Fig. 1 Time course of inflammatory and tissue repair responses after intracerebral hemorrhage (ICH). The onset of ICH causes inflammatory cell death, resulting in the release of proinflammatory factors. Neuronal death occurs rapidly after hemorrhage and continues for the first 3 days posthemorrhage. Expression of factors that regulate the blood–brain barrier (BBB), including matrix metalloproteinases, is first detected at 6 hours post-ICH, and increased BBB permeability is observed for an extended period after hemorrhage. The recruitment of peripheral leukocytes results in significant accumulation of blood-derived macrophages and neutrophils detected at 12 hours post-ICH and peaking at ~24 hours after hemorrhage. At 3 days after hemorrhage, the initiation of pathways involved in tissue repair and hematoma resolution can be detected; however, the duration of these processes remains unclear.

Askenase MH, Sansing LH. Stages of the inflammatory response in pathology and tissue repair after intracerebral hemorrhage. Semin Neurol. 2016; 36: 288-297.

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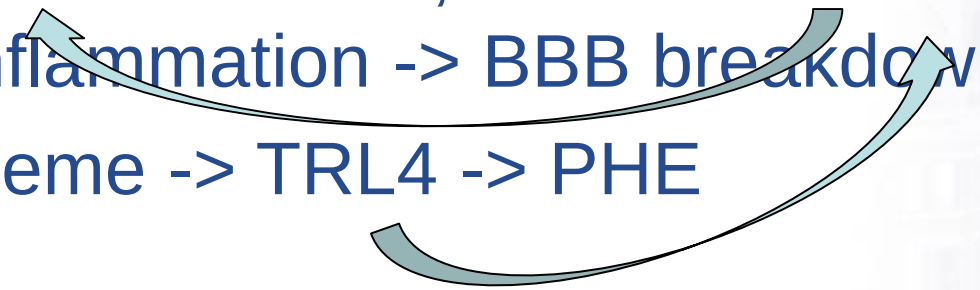
PHE, and Neuroinflammation in ICH

- **Inflammation** (secondary injury) is mediated by:
  - RBC lysis -> Heme, Iron, thrombin -> ROS -> Oxidative stress -> MMP-9 -> PHE
  - ROS -> TNF-α, IL-10 -> NF-κB -> secondary inflammation -> BBB breakdown -> PHE
  - Heme -> TRL4 -> PHE



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8. Aronowski J, Zhao X. Molecular pathophysiology of cerebral hemorrhage: secondary brain injury. Stroke. 2011; 42: 1781-1786.

PHE, and Neuroinflammation in ICH

- Trajectory of early PHE predicts 90 day mortality and functional outcomes
- PHE develops due to Mechanical forces but also **Inflammation**:

Which genetic mediators are at play?

Haptoglobin in Neurologic Disease

- Hp 1-1 genotype was independently associated with better functional outcomes in patients with ICH

10. Murthy SB, Levy AP, Duckworth J et. al. Presence of haptoglobin -2 allele is associated with worse functional outcomes after spontaneous intracerebral hemorrhage. World Neurosurg. 2015; 83, 4: 583-587.

- The Hp2-2 genotype has been associated with worse outcome at 3 months when compared with the Hp1-1 genotype in aSAH

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- The Hp2-2 genotype was associated with higher rates of angiographic vasospasm by transcranial Doppler or conventional angiography performed between day 3 and 14

12. Chaichana KL, Levy AP, Miller-Lotan R et. al. Haptoglobin 2-2 genotype determines chronic vasospasm after experimental subarachnoid hemorrhage. Stroke. 2007; 38: 3266-3271.

- The Hp2-2 phenotype is an independent risk factor for the development of focal and global angiographic vasospasm, and unfavorable outcome

13. Lederc JL, Blackburn S, Neal D et. al.; Haptoglobin phenotype predicts the development of focal and global cerebral vasospasm and may influence outcomes after aneurysmal subarachnoid hemorrhage. PNAS. 2015; 112: 4: 1155-1160

Haptoglobin and its Allelic Frequencies

- Haptoglobin (Hp): an acute-phase reactant, 2 α and 2 β chains,
  - binds extracellular hemoglobin after RBC lysis to prevent ROS formation and increase clearance
  - Three main phenotypes Hp 1-1, Hp 2-1, Hp 2-2
  - Frequencies of Hp1 is reported

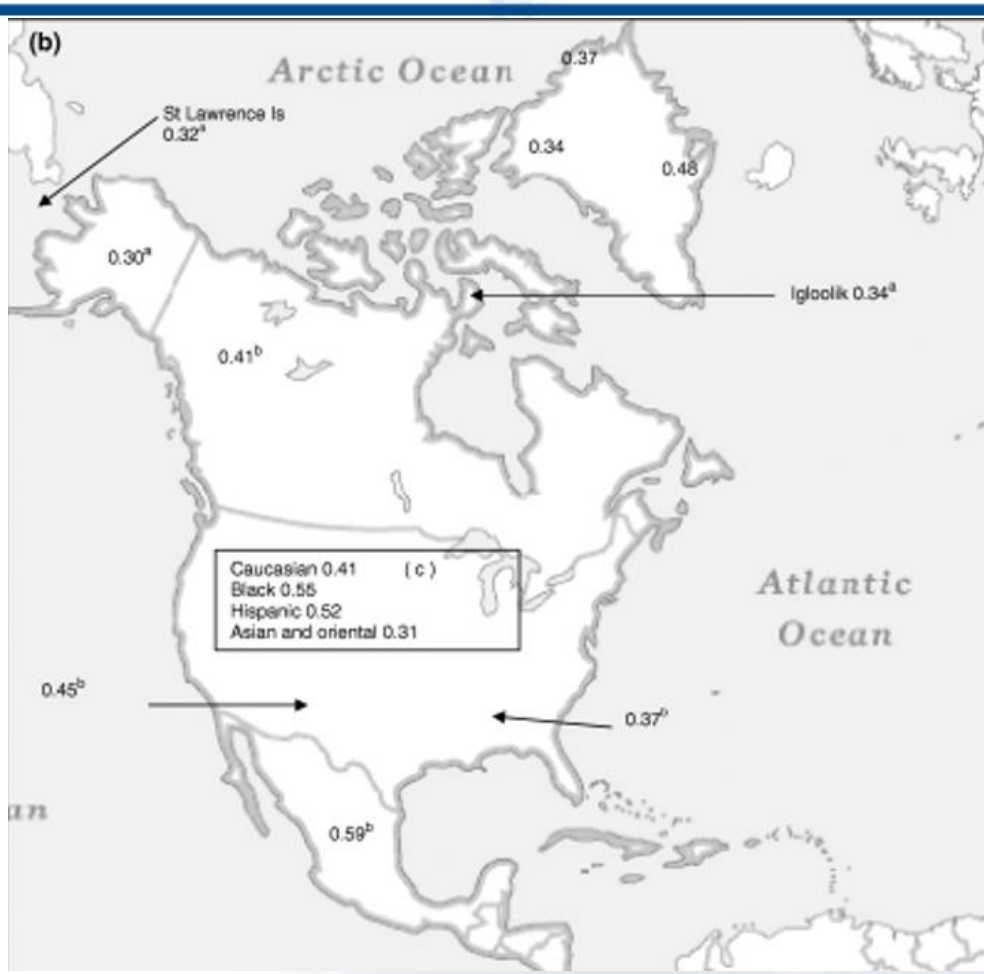

Image curtesy of Paul Nyquist;
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Associations between haptoglobin type and disease. These are case control studies with similar or greater numbers of samples in the control group. Studies with fewer than 50 subjects have not been included. Increased frequency is indicated by ↑, decreased frequency by ↓ and no change by –

Disease type	Specific condition (no. cases)	Hp type or HP[1] frequency			
		1-1	2-1	2-2	HP[1]
Cardiovascular disease	Cardiovascular disease (565)	–	–	–	–
	Coronary heart disease (200)	–	–	–	↑
	Coronary heart disease (297 of 3273)	–	–	–	–
	Coronary heart disease mortality (107)	↑	–	–	–
	Peripheral arterial occlusive disease (141)	–	–	↑	↓
	Essential arterial hypertension (302)	–	–	–	–
	Essential hypertension (257)	–	–	↑	
Cardiovascular disease	Myocardial infarction (121)	–	–	–	–
	Myocardial infarction (496)	–	–	–	–
	Preeclampsia (60)	↑	–	–	–
Inflammation	Rheumatoid arthritis (200)	–	–	–	–
	Family history of polyarthritis (86)				↓
	Haemochromatosis (167)	–	–	↑	–

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Haptoglobin concentrations and phenotype by populations

- Total concentrations vary with phenotype, being lower for Hp2-2 subjects than for Hp1-1 and 2-1
- Values are lowest for the Hp2-1M phenotype (found in African populations)
- Here fewer Hp2 polypeptides are synthesized than Hp1 polypeptides
- However, in Chinese subjects, Hp concentrations were highest for male subjects with Hp2-2

Haptoglobin *α*- and *β*-chain variants detected by electrophoresis listed in order of discovery. Deletions and other mutations associated with ahaptoglobinaemia and anhaptoglobinaemia are listed at http://www.hgmd.cf.ac.uk/. There are population specific base substitutions at the promoter region (Teye *et al.*, 2006

Name	Reported by	Notes
Hp Ca (Carlberg)	Galatius-Jensen (1958)	An α-chain variant with a mixture of Hp2-2 and Hp2-1 bands
Hp2-1M (Modified)	Connell and Smithies (1959)	An α-chain variant encoded by a $Hp\alpha^{2M}$ allele (Giblett & Steinberg, 1960). Maeda found 3 other promoter sequences to explain the variability of the modified phenotype (Maeda, 1991)
Hp2-1 Haw	Giblett, Hickman and Smithies (1964)	An α-chain variant whose pattern shows a high proportion of α^1-chains compared with α^2
Hp Johnson	Oliviero et al., (1985)	A variant with a larger α-chain due to a threefold tandem repeat of a 1.7 kbp DNA segment
Hp1-P & Hp2-P	Robson et al. (1964)	A β-chain variant identified in the presence of haemoglobin by the faster migration of bands, some of which were doubled
Hp1-H & Hp2-H	Robson et al. (1964)	A β-chain variant resembling Hp2-1 and Hp2-2 but with an extra band in the presence of haemoglobin
Hp Mb (Marburg)	Cleve and Deicher (1965)	A β-chain variant resulting from a mutational event causing one or two mutant β-chains
Hp2-1D (Dashing)	Renwick and Marshall (1966)	An α-chain variant from the $Hp\alpha^{1D}$ allele, only distinguishable in the absence of haemoglobin
Hp Ba	Giblett, Uchida and Brooks (1966)	An α-chain variant. Designated 1-B and 2-B when the $Hp\alpha^{B}$ allele is combined with $Hp\alpha^1$ or $Hp\alpha^2$ respectively
Hp2-1 Bellevue	Javid (1967)	A β-chain variant identified by an additional fast moving band in the β-chain region

Estimated Mean Rate of PHE for by Hp Genotype

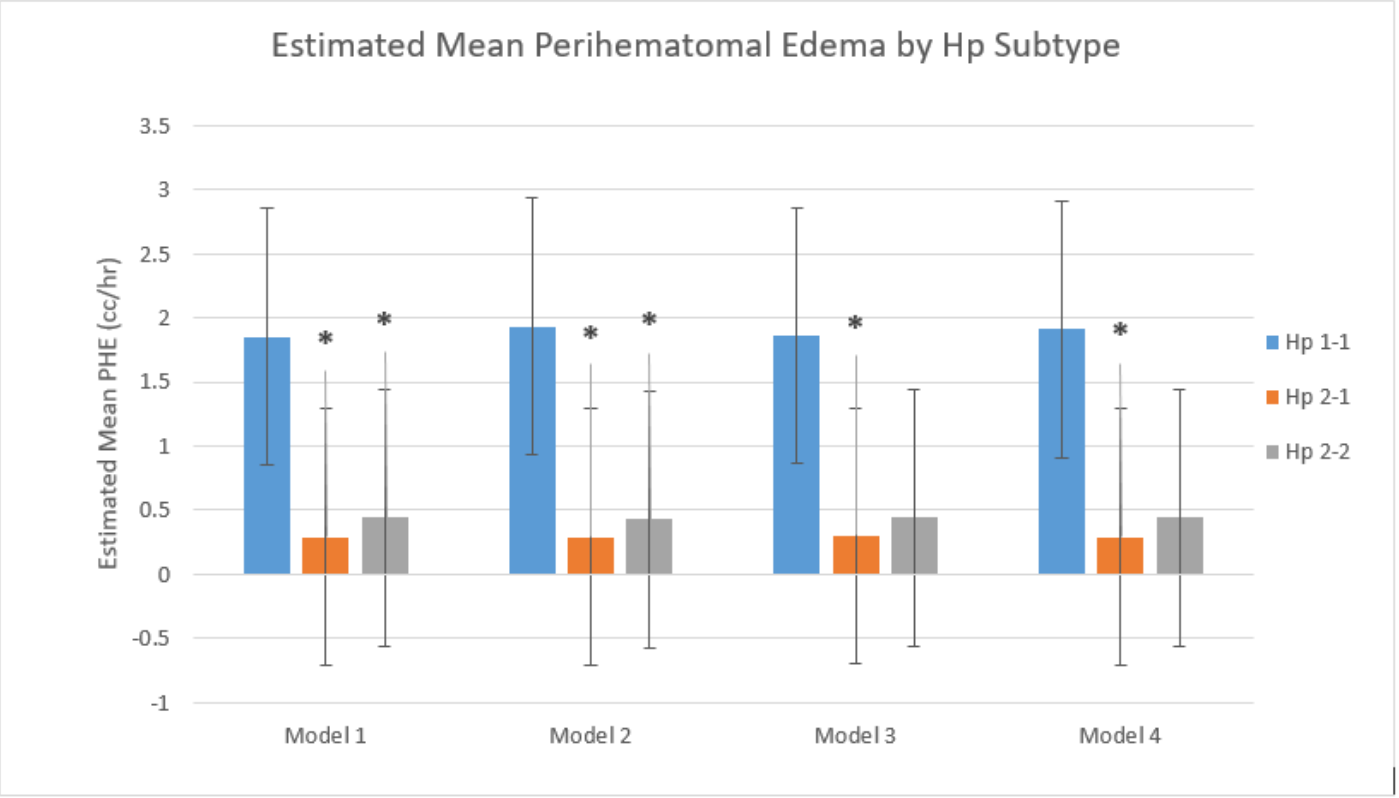

Table 1: Plotted mean PHE by Hp subtype. Brackets represent upper and lower limit 95% confidence interval. Model 1: unadjusted; Model 2: model 1 + age, sex, race; Model 3: model 2 + HTN, HLD, smoking, DM, prior ICH; Model 4: Model 3 + anticoagulation or antiplatelet use at time of presentation. * $p \leq 0.05$, ** $p \leq 0.01$.

Estimated Mean Rate of PHE for by Hp Genotype

	Model 1		Model 2		Model 3		Model 4	
	Estimated Mean (95% CI)	P Value	Estimated Mean (95% CI)	P value	Estimated Mean (95% CI)	P value	Estimated Mean (95% CI)	P value
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Table 2: Adjusted mean PHE by model; Model 1: unadjusted; Model 2: model 1 + age, sex, race; Model 3: model 2 + HTN, HLD, smoking, DM, prior ICH; Model 4: Model 3 + anticoagulation or antiplatelet use at time of presentation

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ICH volumes, Edema Volume, Scan times by Hp subtype

	Genotype	Mean and SD
ICH volume at Time 1	Hp 1-1	29.6 (13.5, 45.6)
	Hp 2-1	19.8 (11.4, 28.2)
	Hp 2-2	18.2 (10.9, 25)
ICH Volume Time 2	Hp 1-1	30.9 (15.2, 45.6)
	Hp 2-1	21.4 (13.2, 29.6)
	Hp 2-2	18.2 (11.1, 25.2)
Edema Volume Time 1	Hp 1-1	25.1 (11.7, 38.6)
	Hp 2-1	18.9 (11.9, 26.0)
	Hp 2-2	17.0 (11.0, 23.0)
Edema Volume Time 2	Hp 1-1	36.1 (19.3, 52.9)
	Hp 2-1	23.5 (14.7, 32.2)
	Hp 2-2	20.2 (12.7, 27.7)
Scan Time	Hp 1-1	21.0 (1.5, 40.6)
	Hp 2-1	20.2 (11.4, 28.9)
	Hp 2-2	23.4 (13.2, 33.6)

ICH, Edema Volumes and Scan time by Hp Genotype.

There was no significant difference between groups

Limitations

- Sample Size – limited overall
  - Underrepresented Hp 1-1 (is this protective?)
- Variability in imaging times
- Medical intervention effect?

Discussion

- Demonstrates an association between early PHE formation and Haptoglobin genotype
- Rates of early PHE in Hp 1-1 were greater than Hp 2-1 and Hp 2-2.

Is this biologically plausible?

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