

Epigenetic approaches in disease studies and some investigated examples

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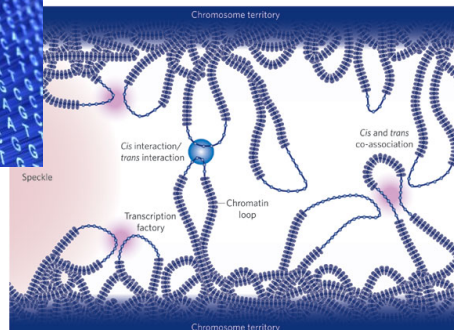
This lecture has been possible thanks to the EU funded Horizon 2020 project, Marie Skłodowska-Curie grant agreement No 713714

REP-eat

How is this information usable to
the living organism?



3% are protein coding genes.
76% transcribed non-coding RNA
21% remaining ... ?
4 million regulatory sites



Celltype-specific...



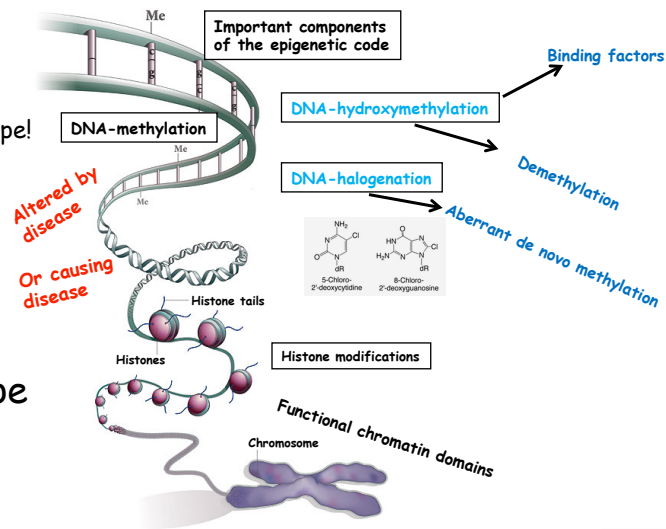
The epigenetic code

The epigenetic code is unique for each cell type!

...likely for each cell

...and for each disease

Association of genotype with epigenotype ?



Epigenetic research?

Why?



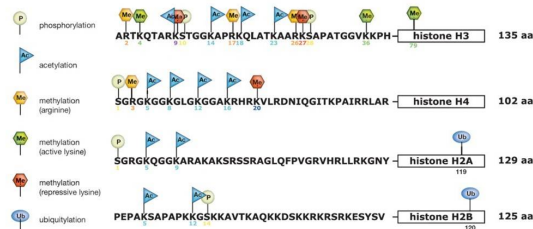
Where in the genome?

1. Promoters
2. "Shore" regions
3. Intergenic regions
4. Intragenic regions
5. Repeat regions
6. Enhancers
7. Euchromatic/heterochromatic borders

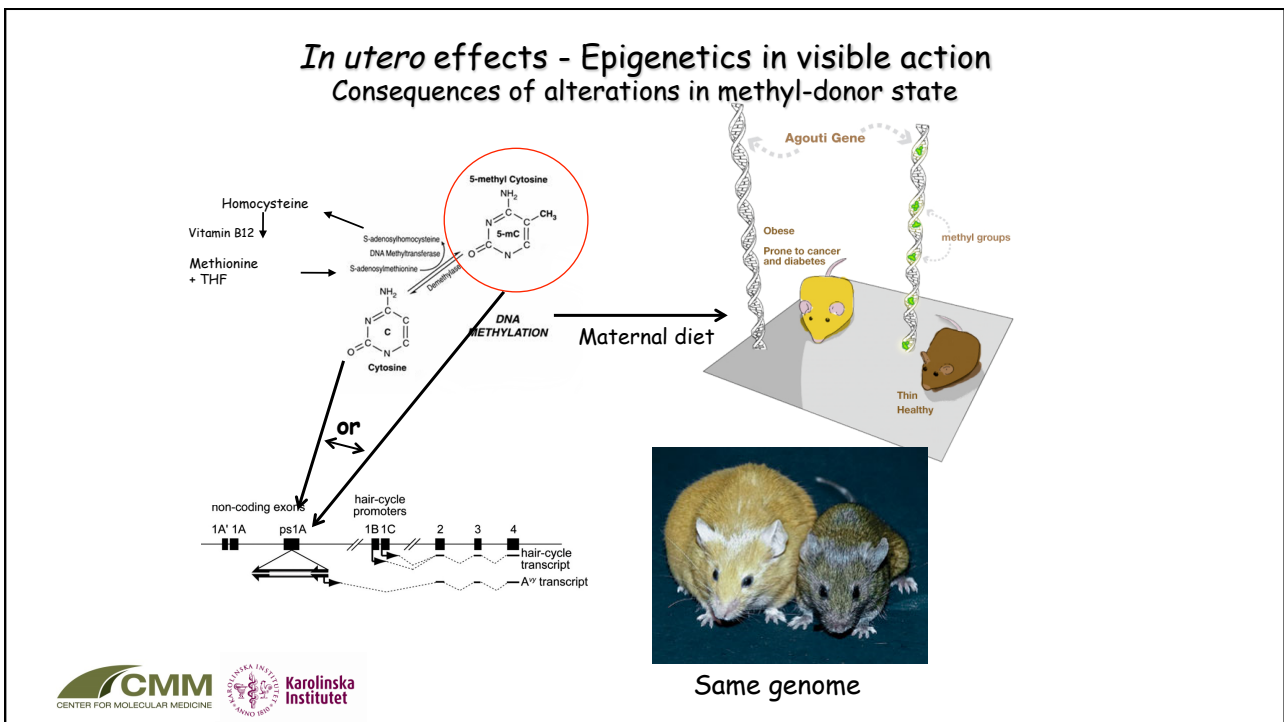
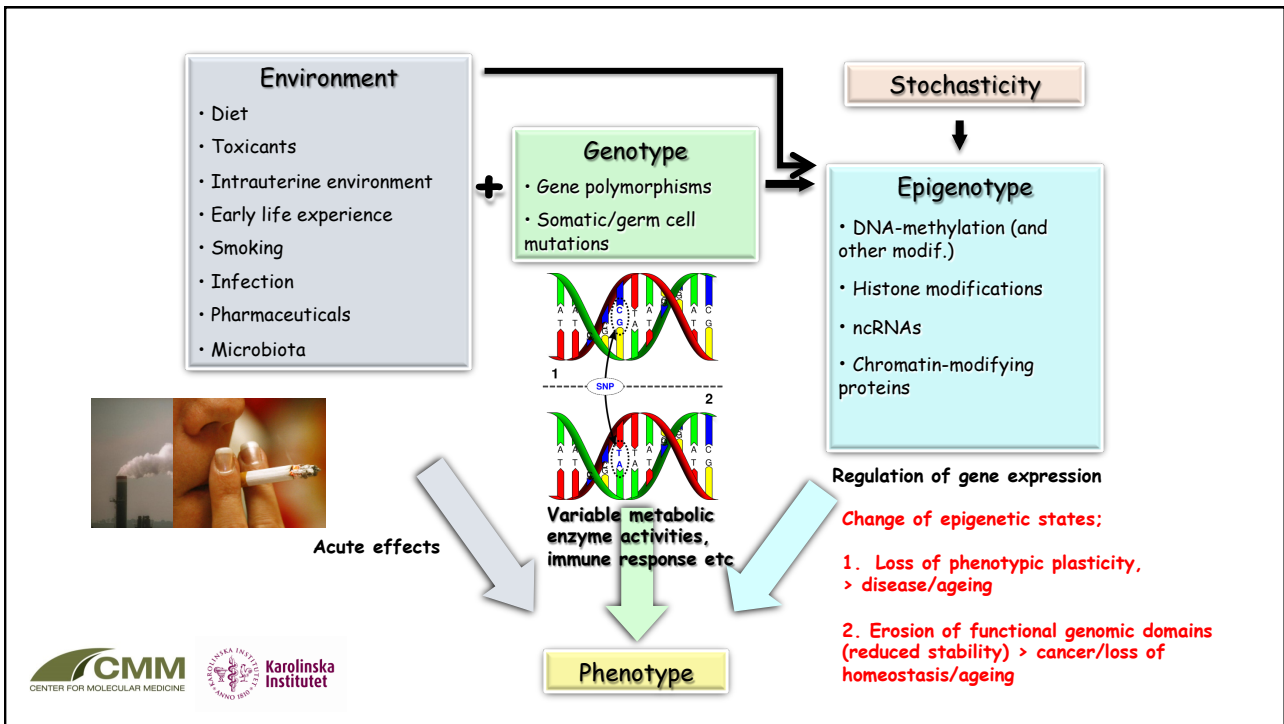
Which modifications?

1. Cytosine methylation
2. Cytosine hydroxymethylation
3. Locus and aa-specific histone modifications, and combinations of these

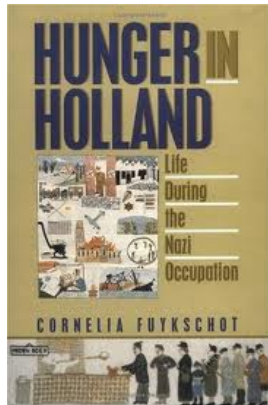
On what clinical samples?



From: www.shechterlab.org/2009/science/chromatin-and-the-histone-code/



The "Hunger Winter" in Holland - *in utero* established epigenetic effects



Dutch famine 1944-45

• Women starving in the first six months of pregnancy gave birth to small babies who grew up to be more prone to obesity, coronary disease, schizophrenia and cancers.

Persistent epigenetic differences associated with prenatal exposure to famine in humans PNAS 2008

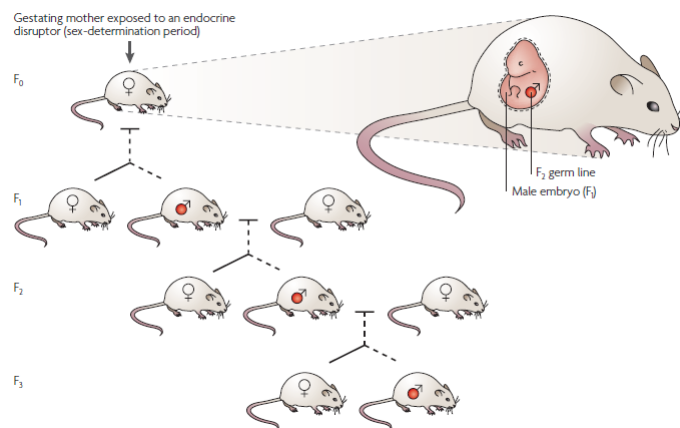
Bastiaan T. Heijmans^{1,2}, Elmar W. Tobin^{1,2}, Aryeh D. Stein³, Hein Putter¹, Gerard J. Blauw⁴, Ezra S. Susser¹, P. Eline Slagboom¹, and L. H. Lumey¹

Departments of ¹Molecular Epidemiology, ²Medical Statistics, and ³Gerontology and Geriatrics, Leiden University Medical Center, Leiden, The Netherlands; ⁴Hubert Department of Global Health, Rollins School of Public Health, Emory University Atlanta, GA 30322; ⁵Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY 10032; and ⁶New York State Psychiatric Institute, New York, NY 10032

Molecular and physiological effects

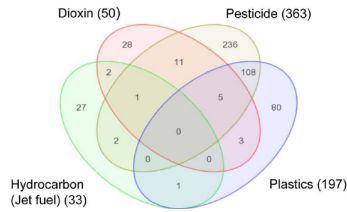


In utero effects - Transgenerational (to F3) effects on epigenetic modification



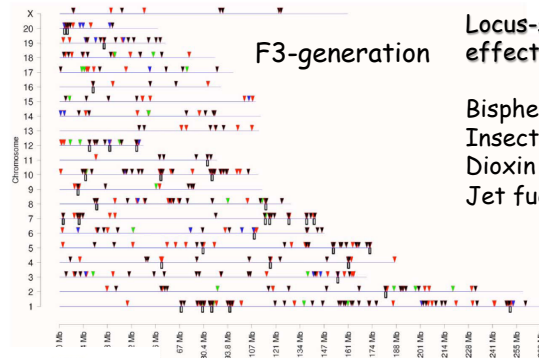
Anway et al. Endocrinol. 2006

Transgenerational differential DNA methylation regions (DMR) associated with exposures



Environmental alterations of epigenotype

B Differential DNA methylation regions (DMR) chromosomal locations



Locus-specific epigenetic effects by xenobiotics

- Bisphenol A (hormone-like)
- Insect repellent (DEET)
- Dioxin (TCDD)
- Jet fuel



Mohan Manikkam et al. Plos One 2012

"Plasticity" via epigenetic mechanisms

Early life effects

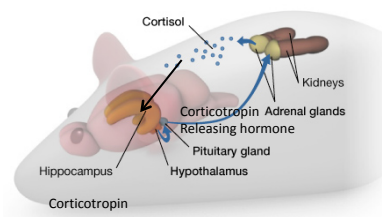
Epigenetic programming by maternal behavior

In C G Weaver^{1,2}, Nadia Cervoni³, Frances A Champagne^{1,2}, Ana C D'Alessio¹, Shakti Sharma¹, Jonathan R Seckl³, Sergiy Dymov³, Moshe Szyf³ & Michael J Meaney^{1,2}



Weaver et al. Nature Neurosci. 2004

HPA-axis



Reversal of stressresponse-related effects by epigenetic drugs

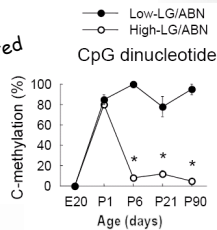


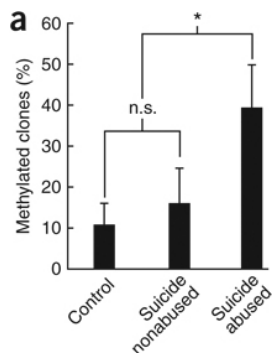
Figure from <http://learn.genetics.utah.edu/content/epigenetics/rats/>

Early life effects: DNA-Methylation in hippocampal GR affected by parental behaviour

nature
neuroscience

Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse

Patrick O McGowan^{1,2}, Aya Sasak^{1,2}, Ana C D'Alessio³, Sergiy Dymov³, Benoit Labonté^{1,4}, Moshé Szyf^{1,5}, Gustavo Turecki^{1,4} & Michael J Meaney^{1,2,5}



McGowan et al. Nature Neurosci. 2009

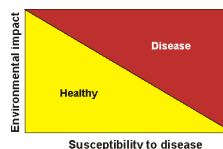
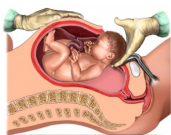
Three strategies for investigating epigenetic factors in disease

Genetics plus environmental factors

Excluding genetic factors
Twins



Long term effects by early and late exposures



Epigenetic analysis

Difficult issues to address:

Causality
Cell type specific epigenomes



Research involving genome-wide methylation analysis

Genetics/epigenetics of rheumatoid arthritis (RA) and multiple sclerosis (MS)
Involvement of smoking

Early life exposures

Epigenetics in chronic alcoholism

Common theme: genetic/environment/epigenetic interactions



Epigenetic basis for autoimmune disease

What is the genetic/epigenetic association/dependency for disease?

Which associations between genome-wide epigenetic profiles, the genotype, and smoking in RA and MS?

How and what to analyze?

Advanced DNA genomewide methylation analysis of blood cells in **large RA and MS cohorts.**

Comprehensive patient clinical/phenotype data



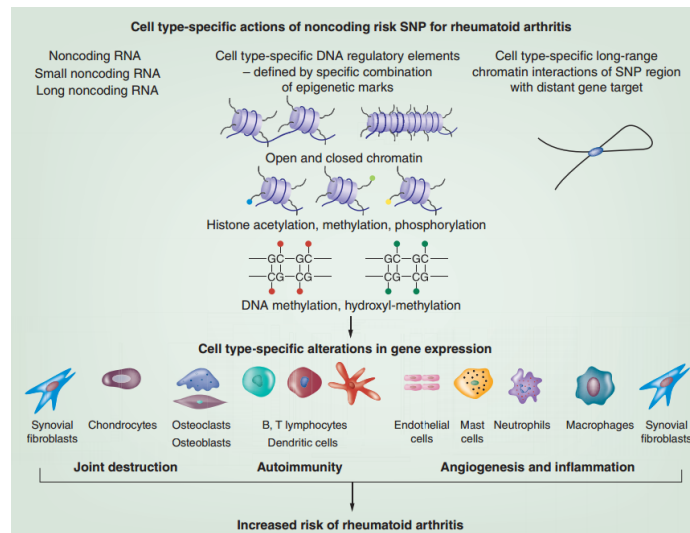
Genetics and environmental factors

Genetics

- DNA methylation associated with disease
- DNA methylation associated/dependent on genotype
- Disease phenotype mediated by DNA methylation alterations
- **Genotype dependent disease phenotype mediated by DNA methylation**
 - N.B. Epigenetic state in disease may be due to variation of cell type

Environment

- Methylation associated with/caused by, environmental impact (smoking)



[Epigenomics](#), 2017 Apr;9(4):493-504. doi: 10.2217/epi-2016-0142. Epub 2017 Mar 21.

From: **Interplay between genetic and epigenetic mechanisms in rheumatoid arthritis.**

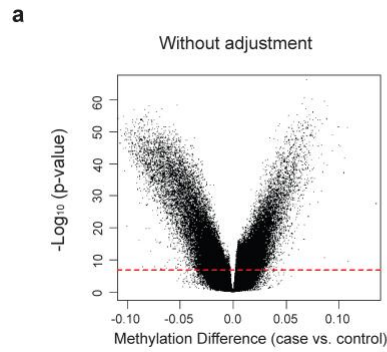
[Frank-Bertoncelli M¹, Klein K¹, Gay S¹.](#)



Genomewide methylation analysis, Illumina Infinium 450k
An ACPA+RA case/control study

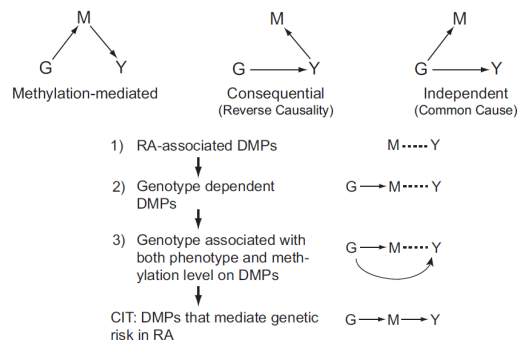
Importance of adjustment for cell type (computed)

RA associated Differentially Methylated Positions DMPs



Liu et al., Nat Biotechnol. 2013

Causal Inference Testing (CIT) for analyzing
genotype (SNP)/epigenotype/phenotype dependencies
(Conditional correlation analysis adjusted to cell type)



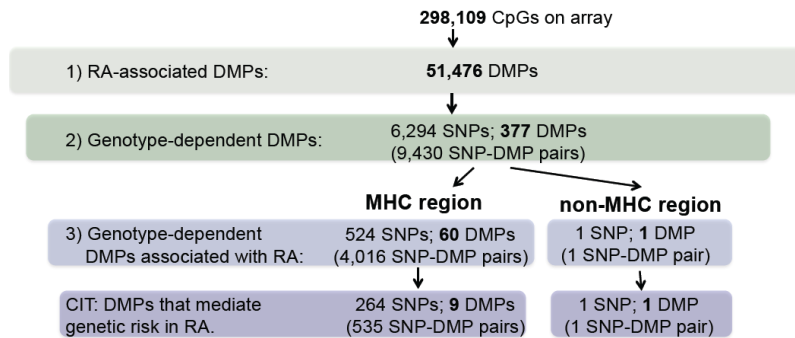
Four components of CIT:

- * G and Y are associated;
- * G is associated with M after adjusting for Y;
- * M is associated with Y after adjusting for G;
- * G is independent of Y after adjusting for M.



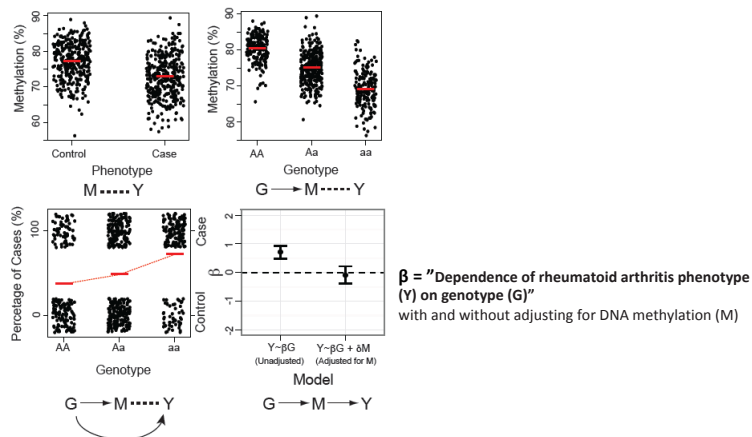
Liu et al., Nat Biotechnol. 2013

Strategy for identifying epigenetically mediated genetic risk factors for RA

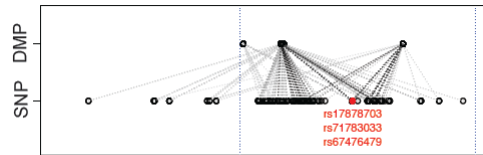


Genotype-dependent DMPs that mediate genetic risk within the MHC region

cg25949002, rs2076540 A DMP, dependent on a genotype (SNP)



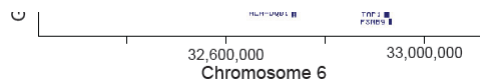
Genotype-dependent DMPs that mediate genetic risk within the MHC region



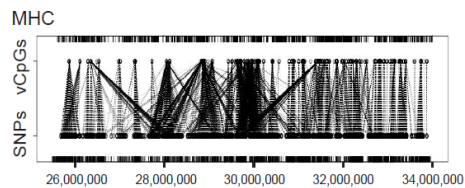
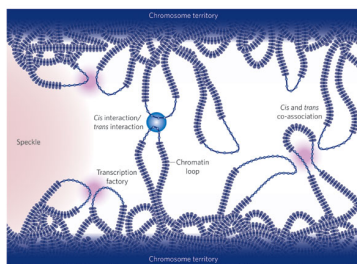
Nat Genet. : 44(3): 291–296. doi:10.1038/ng.1076.

Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis

Soumya Raychaudhuri^{1,2,3,4,†}, Cynthia Sandor^{1,2,3,4}, Eli A. Stahl^{1,2,4}, Jan Freudenberg⁵, Hye-Soon Lee⁶, Xiaoming Jia^{1,4,7}, Lars Alfredsson⁸, Leonid Padyukov⁹, Lars Klareskog⁹, Jane Worthington¹⁰, Katherine A. Siminovitch¹¹, Sang-Cheol Bae⁹, Robert M. Plenge^{1,2,4}, Peter K. Gregersen⁵, and Paul I.W. de Bakker^{1,4,12,13,†}

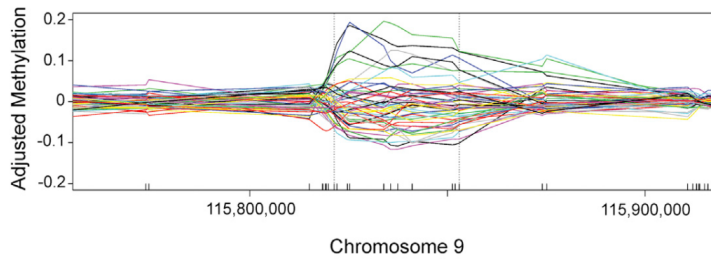


Genetically controlled variable methylation clusters, "GeMes" in healthy individuals



Liu et al. AJHG 2014

Variable methylated regions, VMRs

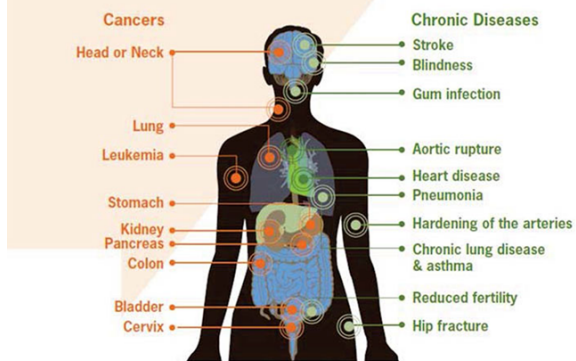


Smoking responsive DNA methylation
is 30-fold enriched in VMRs

Liu et al. AJHG 2014

Risks from Smoking

Smoking can damage every part of the body



and that's not all...

