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## **The Second Draft: The Human Epigenome for novel Diagnoses and Therapies**

*“The major problem, I think, is chromatin... you can inherit something beyond the DNA sequence. That’s where the real excitement of genetics is now”*  
– James Watson

The massive sequencing effort of the Human Genome Project spanned over thirteen years (1990-2003) and cost up to an estimated of three billion U.S. dollars. However, the results were significant. The advancements in mapping out this ‘first draft’ of the human genome have facilitated the acquisition of knowledge of the effects of variation of DNA among individuals. This then revolutionized the ways modern medicine diagnoses, treats and even prevents many diseases.

However, it appears that now, DNA does not translate to destiny because of a second draft – the human epigenome. What exactly is the epigenome or epigenetics? Epigenetics was first described to be the bridge between genetics and developmental biology – the concept that mechanisms in the former could influence expressions in the latter. Since then, the discovery that 5-methyl histone has a role in gene expression and the discovery that the pattern of methylation is heritable to silence particular gene expressions without changing the coding sequence have altered the view that chromatin (the nucleoprotein complex of DNA and histones) is a non-dynamic structure.

Now it has come to light that the epigenome – the second level that interprets the blueprint that is the human genome – sits on top of your genome and regulates the gene expression and then by extension the phenotypes displayed. Hence, with the new

discoveries of DNA methylation, along with post-translational histone modifications and the various roles of microRNA, new clinical applications in diagnoses and therapies arise.

So far, the advancements in epigenetics have benefitted the following areas:

**(1) Genome annotation**

Many recent ENCODE publications show that chromatin signatures enable more efficient and more precise genome annotation of regulatory elements. With the genome annotation on this second level of gene expression it is more possible to pinpoint functional or cell type-specific regions in studies.

**(2) Cell Identity**

Epigenomic maps can also provide more information than simple gene expression data. With epigenetics, it is possible to deduce chromatin states regardless whether genes are either expressed or not. The new information refines our understanding of the activity status of genes such as whether it is primed or poised or in varying stages of repression. This additional information cannot be obtained through simple genetic or gene expression measure.

However, the most exciting significance of the new developments of epigenetics is the light it sheds on complex human disease. This came to be because multicellular organisms require the mutually reinforcing mechanisms that permit heritable patterns of gene silencing. Some of the mutations in these genes that affect epigenetic profiles across the globe result in human disease that are inherited via the methylation patterns or *de novo* somatically acquired. It is now known that only about 2% of human diseases such as anemia and cystic fibrosis are simple Mendelian, the rest, such as schizophrenia, autism, diabetes etc. are complex non-Mendelian that epigenetics have helped better understand

It can be seen that the understanding of epigenetics is the key to understanding the genetic and epigenetic basis of so many more complex disease. The table below highlights some epigenetic diseases that have been more clearly understood due to recent discoveries.

Table 1 Epigenetic diseases			
Disease	Symptom	Aetiology	References
ATR-X syndrome	Intellectual disabilities, $\alpha$ -thalassaemia	Mutations in <i>ATRX</i> gene, hypomethylation of certain repeat and satellite sequences	82
Fragile X syndrome	Chromosome instability, intellectual disabilities	Expansion and methylation of CGG repeat in <i>FMR1</i> 5' UTR, promoter methylation	83
ICF syndrome	Chromosome instability, immunodeficiency	<i>DNMT3b</i> mutations, DNA hypomethylation	84
Angelman's syndrome	Intellectual disabilities	Deregulation of one or more imprinted genes at 15q11–13 (maternal)	85
Prader-Willi syndrome	Obesity, intellectual disabilities	Deregulation of one or more imprinted genes at 15q11–13 (paternal)	86
BWS	Organ overgrowth	Deregulation of one or more imprinted genes at 11p15.5 (e.g. <i>IGF2</i> )	87
Rett syndrome	Intellectual disabilities	<i>MeCP2</i> mutations	25,26
$\alpha$ -Thalassaemia (one case)	Anaemia	Methylation of $\alpha 2$ -globin CpG island, deletion of <i>HBA1</i> and <i>HBA2</i>	23
Various cancers	Microsatellite instability	<i>De novo</i> methylation of <i>MLH1</i>	29
	Disruption of Rb, p53 pathway, uncontrolled proliferation	<i>De novo</i> methylation of various gene promoters	4
	Disruption of SWI-SNF chromatin remodelling complex	Mutations in <i>SNF5</i> , <i>BRG1</i> , <i>BRM</i>	36
	Overexpression of <i>IGF2</i> , silencing of <i>CDKN1C</i>	Loss of imprinting	88, 89
Leukaemia	Disturbed haematopoiesis	Chromosomal translocations involving HATs and HMTs	62
Rubinstein-Taybi syndrome	Intellectual disabilities	Mutation in CREB-binding protein (histone acetylation)	90
Coffin-Lowry syndrome	Intellectual disabilities	Mutation in <i>Rsk-2</i> (histone phosphorylation)	90

ATR-X syndrome,  $\alpha$ -thalassaemia, mental retardation syndrome, X linked; BWS, Beckwith-Wiedemann syndrome; CREB, cAMP-response-element-binding protein; HAT, histone acetyltransferase; HMT, histone methyltransferase; ICF, immunodeficiency, centromeric region instability and facial anomalies syndrome; UTR, untranslated region.

From this list I have isolated Fragile X Syndrome and the various cancers to evaluate as examples of the progress made through epigenetic findings. The symptoms that define Fragile X syndrome are very much related to the mutations in the Fragile X Mental Retardation gene 1 (*FMR1*) which, according to my search on UniProt, functions as a translation repressor. It is now known that a common form of inherited mental retardation related to the *FMR1* gene results when the repeat of CGG codon in the *FMR1* 5' untranslated region undergoes expansion and becomes methylated *de novo*. This causes the gene to be silenced and creates the visible 'fragile' site on the X chromosome in some cases. It is evident that the understanding of the development of Fragile X syndrome allows confirmation of disease through checking for the indicators such as the methylation of the repeat of CGG.

I also focused on cancer. From the table it is evident that detection of *de novo* methylation, mutations and loss of imprinting could all be indicators for cancerous

1 Egger, Gerda, Gangning Liang, Ana Aparicio, and Peter A. Jones. "Epigenetics in Human Disease and Prospects for Epigenetic Therapy." Nature.com. Nature Publishing Group, n.d. Web. 03 Dec. 2012. <<http://www.nature.com/nature/journal/v429/n6990/full/nature02625.html>>

diseases. One specific example is prostate cancer: there are potential clinical applications of epigenetic management. For example, markers of DNA hypermethylation are subject to many studies as potential complementary diagnostic tools, prognostic factors, and predictors of responses to treatment. An example is the detection of *GSTPI* methylation to distinguish between prostate cancer and the benign process. The *GSTPI* gene is hypermethylated in 80-90% of patients with prostate cancer, but not in benign hyperplastic prostate tissue. Hypermethylation of CpG islands can then be used as a marker of cancer cells in all the different types of biologic fluids and biopsy specimens, thus making the detection of *GSTPI* methylation in urine a possible clinical application of diagnosis.

With these diagnoses, the race now is to find the drugs that can reverse the gene silencing that occurs at the epigenetic level. There now are new clinical trials that have been looking into targeting the reversal of DNA methylation and histone acetylation. According to my research, The prototype inhibitors of DNA methylation, 5-azacytidine (5-aza-CR) and 5-aza-2'-deoxycytidine (5-aza-DdR), seem quite promising with clinical trials achieving phase I/II/III. However, the development of this inhibitor is still tentative along with many other drugs in development right now. 5-aza-DdR, has so far been observed to be a potent antineoplastic agent against leukemia and tumors in laboratory animals. This antineoplastic activity has also been seen in patients with leukemia, myelodysplastic syndrome (MDS) and non-small cell lung cancer (NSCLC) in preliminary clinical trials of the 5-aza-CdR.

Many trials are now also trying to reverse histone acetylation. It has also been suggested that coupling these therapies – DNA methylation inhibition and histone acetylation reversal has potential in therapeutic treatments.

It is evident that epigenetics, this second draft of the human genome, has allowed for complex diseases to be further investigated and novel diagnostic tools and

therapies to be developed. The Human Epigenome Project, which ‘aims to identify, catalogue and interpret genome-wide DNA methylation patterns of all human genes in all major tissues’ seems to respond to the potential in this field.

The potential advancements in the Human Epigenome Project (HEP) could facilitate the establishment of Epigenome Wide Association Studies (EWAS) on top of the Genome Wide Association Studies (GWAS) that resulted from the findings of the human genome project. There have already been papers published that envision the use of case-controls, parent-offspring pairs, monozygotic twins and longitudinal cohorts as samples and perhaps the new disease-risk epigenetic markers that may be defined by the HEP in providing these useful studies on complex diseases.

So why is it that HEP has not escalated to the scale that the HGP had over those thirteen rigorous years? At the moment, it appears that the project is solely funded by European and American agencies: The Wellcome Trust Sanger Institute (UK), Epigenomics AG (Germany/USA), The Centre National de Génotypage (France). Is it because it might just be another one of those academic exercises?

From my research and what I have interpreted as the implications of future epigenome research, it appears that the human epigenome project is not just another exercise in number crunching, mechanism hunting, and code mapping. It has been argued that the epigenome is inherently important in redefining the direction of medical diagnostics and therapies even beyond what the human genome project has done. The human genome is the blueprint of life, however, the human epigenome that I have defined as the ‘second draft’, is not only a second layer of code, but a dynamic, reversible web of mechanisms that the scientific community can manipulate in order to advance medical diagnoses and therapies.

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