TAILORING BIOTECHNOLOGIES
Vol. 3, Issue 2, Summer 2007, pp: 41-48

The End of Disorder: a case of premature termination and PTC124.

Steve Hughes

In a Nature article entitled *PTC 124 targets genetic disorders caused by nonsense mutations,* Welch and a large group of co-workers present evidence that a simple chemical of the oxadiazole benzoic acid family (PTC124) can suppress the consequences of some otherwise devastating mutations in human genomes (Welch et al 2007). If confirmed in the pharmaceutical context this could mark a turning point in the management of disease and in the way in which we view human genetic variation in relation to the concept of disorder.

The term disorder seems to carry a commonplace sense in relation to our individual maladies in particular those of the chronic persuasion. By its connotation with concepts of social conditions of institutional breakdown, anarchy or topsy turviness, it seems to imply a maladaption or maladjustment to the environment or culture within which we live when we are compared to the normal. That is to say that our personal order does not fit with or match the ambient order of things. It also seems to align with some state with which we are irreversibly endowed rather than a condition which we might transiently acquire, such as an infectious disease, though this is not always so, especially in the world of mental health where the term disorder seems to be generally preferred to disease[1]. Nevertheless, the term seems to fit very well in common usage with genetic departures from the ordinary as in "genetic disorder" that set of consequences of genetic determinants or predispositions generally inherited but sometimes acquired in the reproductive process e.g. trisomy as linked to Downs Syndrome. Thus a disorder assigns us a status for our lifetimes which means that society has to make individual and by implication costly concessions in order to accommodate us, generally via the healthcare and welfare systems. A disorder is unambiguously part of our identity in a way in which illness or disease, which may be episodic, are not.

1 Presumably because altered mental function and minority behavior patterns are more clearly associated with disruption of order in society. A Google internet search using the terms "disorder criteria" lists innumerable sites all related to mental disorder.

Let me think this through in relation to one of my own genetic disorders, that of being left handed.

A minority, around 10% of us, are either through inheritance or congenital effects left handed. This marks us for life as an outgroup, sinister, disadvantaged and maladapted to a culture whose artefacts, language and styles of doing are shaped for the right handed. There is potential for left handers to kill or maim themselves or others by the use of machines designed for use by the right handed. We are truly a source of disorder. And yet, left handedness is not generally regarded as a disorder, I would argue, because by diligence we manage to compensate and to sustain a reasonable quality of life without huge cost to the right handed majority. Alternatively it may be that in a society in which there is extensive division of labor there is collective benefit from having a diversity of capabilities and that those special capacities which accrue from left handedness are drawn in to the overall capacity of the society, the wealth of the nation.

In contrast few would argue with the inclusion of phenotypes (manifestations) such as muscular dystrophy, cystic fibrosis, Huntington's or Down's Syndrome in the list of genetic disorders[2].

So perhaps I'm now in a position to disentangle some of the criteria employed in the concept of disorder in general usage, against which to develop my critique.

1. it inevitably assigns the status of an outgroup to those who manifest the phenotype: in the extreme this can be oppressive and socially discriminatory[3]
2. the phenotype confers a serious challenge to quality of life: in the extreme this can be distressing, disabling, painful and life threatening.
3. the consequences of the phenotype place a calculable cost on carers and society in general; in the extreme this can mean that adequate support cannot be provided by the state and specific campaigning charities take over part of the role.

These interlocking criteria are undeniably socially and economically constructed and in this context it is interesting to ask how there can or could be an

2 In fact each of the organisations representing these phenotypes are members of the Genetic Interest Group in the UK which uses the term genetic disorder in its terms of reference

3 rationale for the phenomenon of exclusion, the use of disorder to support exclusion, and the use of exclusion to assign disorder is a complex and variable field and well beyond a fitting coverage in this article. The discourse is anchored on works such as Witchcraft and Leprosy: two strategies of exclusion by Mary Douglas and The elements of Social Theory 1995 by Barry Barnes

end to disorder. Francis Fukayama predicated the End of History on the demise of one of the partners to the major conflict of modern history. Can we do the same for disorder by demobilizing one or more of the above criteria? Can this be achieved only by social renegotiation or can scientific advance play a part in the de-categorization of disorder and the self-assignment of identities?

One approach has been to "take out"[4] the outgroups in the crudest possible sense, basically by blocking recruitment to them by enforced or coerced sterilization or by application of prenatal diagnosis and abortion. While still somewhat short of a coherent eugenic movement this approach has its practitioners, its vocal proponents and its cautious critics. Take for example the campaign mounted to render accountable the abortion of a hair lip/cleft palate-prone foetus[5]. In fact this amounted to a move to invalidate the disorder by bringing in the outgroup, and represents an interesting case of an assertion of the right to negotiate identities in relation to genetic predispositions. A continuation of this movement could bring an end to disorder.

The demobilization of the third criterion requires a change in social attitude based on the recognition that we all impose a cost on our families and on society generally. The overall calculus of cost and contribution across lifetimes is complex and, sensibly, is not individualized. That is, unless we are part of an outgroup associated with a disorder, in which case the costs are highlighted and the contributions ignored.

The response to the second criterion is to alleviate the phenotype. The classic early example is phenylketonuria (PKU) where early post-partum screening can indicate the need for a diet depleted in phenylalanine[6]. As a consequence of universal testing PKU is anticipated by a change in diet and is no longer a disorder. The social cost of testing is small compared to the costs of caring for affected individuals were PKU still a disorder.

Another example is blood typing of the rhesus and ABO classes. Although this defines groups or medical categories differentiated as universal donors or universal recipients (advantaged, disadvantaged) or by risk to the unborn, they only give rise to phenotypes during the need for a blood transfusion or during pregnancy. In either case the phenotype is overcome by appropriate treatment coupled to the socially motivated donation of blood to members of one's own type. Blood types are not considered as disorders or used to define outgroups.

4 "take out" is here used in the Schwarzeneger sense: eliminate

5 see http://www.prolife.org.uk/show?item=189 http://www.telegraph.co.uk/opinion/main.jhtml?xml=/opinion/2005/03/20/do2001.xml&sSheet=/opinion/2005/03/20/ixop.html

6 see http://www.hta.nhsweb.nhs.uk/execsumm/SUMM111.HTM

Latterly in this vein of phenotypic alleviation a very interesting prospect has appeared which to use Feenberg's critique of technology, may root itself in the technical code related to the socially desirable goal of ending disorder (Feenberg 2005). A company called PTC Therapeutics Inc (Post Translational Control) has announced the discovery and preliminary testing in vitro and in animal models of a simple compound PTC 124[7] which can potentially alleviate the phenotypes of a range of genetic disorders (Welch et al 2007). It does so by overriding (suppressing) a set of mutations which give rise to premature termination of transcription in the process of protein synthesis. Such mutations are prominent among loci corresponding to genetic disorders including the aforementioned cystic fibrosis and muscular dystrophy and they are otherwise known as nonsense mutations. They are differentiated from the other class of point mutations (mutations introduced by single base-pair changes =SNPs) occurring in coding sections of the genome, known as mis-sense. Nonsense mutations may arise by a single base substitution at any one of 23 of the 61 possible coding triplet templates corresponding to 8 of the possible amino acids, creating an inappropriate stop signal for protein synthesis. Whereas mis-sense mutations result in the incorporation of an inappropriate amino acid (substitution) in the growing protein chain during translation of the RNA template, nonsense mutations result in a termination of the translational process and rapid breakdown of the RNA template thereafter. In terms of products, mis-sense leads to an altered site in the protein which may range from neutral to fully inactivating (loss of gene function) in its effect. Nonsense leads to the production of a truncated protein, which, even if it has some residual activity, is produced in reduced amounts owing to the compounding effects of RNA breakdown.

The suppression of chain termination

The template relationship between the sequence of bases in DNA, the intermediate template messenger RNA and the sequence of amino acids in the corresponding protein depends upon a complex set of components forming the protein synthetic machinery the function of which is controlled by start and stop signals implicit in the genetic code. Mutation can give rise to inappropriate stop signals which uncouple the synthetic process.

7 1,2,4-oxadiazole benzoic acid compounds together with a large family of related compounds, all subject to a suite of patents granted and pending

Such events are termed nonsense mutations and the uncoupling process chain termination. The characteristics of nonsense mutations, premature chain termination and its suppression were first explored in micro-organisms (bacteria and bacteriophages) Each of the classes of nonsense, amber (where an amino acid encoding triplet is mutated to a UAG translational stop signal), ochre (stop code UAA) and saffron or opal (stop code UGA) were found to have pre-existing corresponding suppressor loci in some strains of bacteria, meaning for instance, that many amber mutations had no phenotype in amber suppressing strains. It seems that the original isolate of E.coliK12 which subsequently became the universal laboratory model organism either carried nonsense suppressors or acquired them early in its lineage[8]. There are a range of suppressors for each of the stop signals each leading to the insertion of a specific amino acid and suppressors are a common discovery in new isolates of bacteria[9]

The correspondence of nonsense and suppressor was fortuitous for microbial genetics since it permitted the analysis of functions for which lethal mutations could not be restored nutritionally. Conditional lethal mutations of this type i.e. only lethal in absence of a suppressor, played a significant role in the analysis of functions such as DNA replication, the synthesis of other cellular macromolecules and the functions encoded in bacteriophage genomes.

Suppression of nonsense, then, is not a new concept or reality. In the case of bacteria it is commonplace and found to be attributable to a sub-population of transferRNA species endowed with the ability to recognise the stop codon triplets and to present an amino acid for insertion at that site. This allows "read through" of the messenger RNA and, to extend the linguistic metaphor, the over-riding of the nonsensical form of aberrant punctuation.

An early application of conditional mutations of the above type in commercial biotechnology is discussed by Hughes and Brown (1973).

The suppression of the effects of nonsense mutations by PTC124 and its related compounds is attributed to its ability to recognize and attach itself to a molecular signature in the protein synthesis machinery. The component it

8 Belin 2003

9 Marshal and Levy 1980

interacts with is called the ribosome which is itself a complex assembly of functional units which acts as a mobile scaffold to support the sequential addition of amino acids to the growing protein chain against the RNA template. Like the alternative tRNAs of bacterial suppression it supports read through at the stop signals of nonsense mutations and prevents both premature termination and associated RNA breakdown. The really interesting observation is that in the range of genetic scenarios so far examined it does not interfere with the correct stop signals at the ends of protein coding sequences. This removes one fear of possible side effects when PTC124 is used in vivo.

So far so good, the compound is readily ingested but much testing and evaluation remains to be done before it can become part of the diet . However, if all goes well and the compound is able to suppress a range of nonsense mutations across a range of genetic disorders[10], a whole set of outgroups will be split down the middle dependent upon whether their particular genetic variant is nonsense or mis-sense. For the nonsense cohorts there will be an end to disorder and PTC124 will acquire the status of a vitamin provided that monopoly and market forces do not price it out of reach of the common person. Nevertheless, we can foresee the emergence of a market which will drive investment in exploration of further drugs of the type.

PTC124 raises some intriguing questions with respect to the prevalence of chain terminator mutations in the population at large. Do we all carry one or more of them hidden or rendered permissive within our phenotype by lack of ambition and willingness to opt for a cushy lifestyle? Could we all have a slightly fitter existence and develop new potential with the help of PTC? This is to oversubscribe to genetic essentialism but it helps to make the point that while PTC124 could help to deconstruct disorder it could also help first to construct and then deconstruct the disorder that we didn't know that we didn't know we had. The potential market grows, though this "Brave New" projection of chemical gene therapy and human advancement needs to be offset by cautious consideration of potential for epigenetic side effects. For instance, might some pseudogenes[11] be reactivated and bring about inappropriate protein production causing confusion for our cells? We are increasingly aware of the

10 there are a lot of possibilities: Atkinson and Martin in 1994 had already identified and listed 880 nonsense mutations at human disease loci recorded in databases well before human genome sequencing project was completed. Today the Online Mendelian Inheritance in Man (OMIM) site (http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM) and the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) have a tally of over 30,000

11 the term pseudogene refers to DNA sequences which exhibit most of the features necessary for them to act as functional templates for RNA and protein synthesis but are rendered silent by small or large discontinuities. These can include nonsense mutations.

importance of small RNA molecules derived from mRNA, RNA trans-splicing and RNA turnover as "ordering" mechanisms in the control of gene expression and cellular development. It is not impossible that nonsense suppression could cross react with this complexity producing unacceptable side effects in which case while losing a potentially therapeutic agent we shall learn more about the intricacies of genetic regulation just as we did from the suppression of nonsense mutations in bacteria.

PTC 124 could find utility beyond the human pool of genetic diversity. The plant and animal varieties on which we depend may similarly carry cryptic chain terminator mutations which render them slightly suboptimal dependent upon their environmental conditions. Perhaps a further generation of nonsense suppressor molecules will be agrochemicals. In that context they might even be used to reactivate deliberately nonsense-silenced transgenes to moderate productivity options e.g a switch between food use and non-food use (energy crop) in response to market fluctuations.

Even if PTC124 falls short of this optimistic expectation it has highlighted the potential of scientific discovery to confound discriminatory categorisations like disorder. PTC 124 reveals the genetically subtle though functionally profound difference between mis-sense and nonsense mutations in our DNA. Material DNA sequences per se (markers based on DNA polymorphisms) as well as their increasingly nuanced relationship to phenotypes and hence to the construction of identities, will doubtless continue to provide a platform for discourses around genetic essentialism, bodies and changing identity politics for some time yet. Whether this be in the context of individual identities, and the assignment of guilt or innocence via forensic tests, the classification of healthcare needs, the self-identification and cohesion of "at risk" groups, or the reassessment of ethnic, cultural or even sexual assignments, a full and proper discussion is beyond the scope of this article. However, in the narrower context of disorder and human and relations perhaps in the light of PTC124 now is a good time to reconsider and "dis" the term in relation to genetic diversity. Let us continue the work of bringing in the outgroups disorder creates by prioritising the discourse on infrastructures and attitudes and by accepting that mutations are ubiquitous and normal and whatever the associated phenotype may be, tendencies to discrimination based upon them should be suspended in the face of the challenge from science and technology to our over-simplified categorisations.

References

Atkinson, J. and Martin, R. (1994). Mutations to nonsense codons in human genetic disease: implications for gene therapy by nonsense suppressor tRNAs, Nucleic Acids Research 22 1327-1334

Barnes, B. (1995). The Elements of Social Theory, UCL Press London ISBN 1-85728-204-3

Belin, D (2003). Why Are Suppressors of Amber Mutations So Frequent Among *Escherichia coli* K12 Strains?: A Plausible Explanation for a Long-Lasting Puzzle 1995 Genetics 165 455-456

Douglas, M (1991). Witchcraft and Leprosy: two strategies of exclusion, Man, New Series, 26, No. 4. 723-736.

Feenberg, A. (2005). Critical Theory of Technology. In: Ruivenkamp, G. and J. Jongerden. 2005. Potentialities, Actualities and Spaces. Volume 1, Issue 1 *Tailoring Biotechnologies.* Wageningen-Istanbul.

Hughes, S.G., and Brown, P.R. (1973) Improved preparation of bacteriophage T4-infected Escherichia coli for the isolation of polynucleotide kinase, Biochem.J. 131,583

Marshal, B. and Levy, S.B. (1980). Prevalence of amber suppressor-containing coliforms in the natural environment. Nature 268 524-525

Welch, E.M. E. R. Barton, J. Zhuo, Y. Tomizawa, W. J. Friesen, P. Trifillis, S. Paushkin, M. Patel, C. R. Trotta, S. Hwang, R. G. Wilde, G. Karp, J. Takasugi, G. Chen, S. Jones, H. Ren, Y-C. Moon, D. Corson, A. A. Turpoff, J. A. Campbell, M. M. Conn, A. Khan, N. G. Almstead, J. Hedrick, A. Mollin, N. Risher, M. Weetall, S. Yeh, A. A. Branstrom, J. M. Colacino, J. Babiak, W. D. Ju, S. Hirawat, V. J. Northcutt, L. L. Miller, P. Spatrick, F. He, M. Kawana, H. Feng, A. Jacobson, S. W. Peltz1 & H. L. Sweeney (2007). PTC124 targets genetic disorders caused by nonsense mutations Nature 447, 87-91