# *"Information is the resolution of uncertainty"* Whole Genome approaches to genetic diagnosis on the PICU.

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**Key words**
Whole genome sequencing, critical illness, whole exome sequencing, diagnosis, rare disease

This work received no direct funding but was supported by the National Institute for Health Research Great Ormond Street Hospital Biomedical Research Centre. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the UK Department of Health.

**Declaration of Interests**

No conflicts of interest to declare.

Word count 1000

Sherwin B. Nuland, surgeon and bioethicist wrote "*To become comfortable with uncertainty is one of the primary goals in the training of a physician"*. (1) Whether we are comfortable with it or not, uncertainty is a feature of our work. We are rarely certain about the risk and benefits of therapy, we struggle to estimate prognosis and, often, diagnoses are elusive. We use clinical and laboratory information to try to reduce this uncertainty.

In this context is it worth remembering what '*information'* means formally. Claude Shannon, a mathematician and the father of Information Theory, defined it as '*a measure of the number of possible alternatives*.' (2) The 'bits' that we count in their billions in our devices are choices between two states: '0 and 1' or 'true' and 'false'. The bit is therefore the lowest possible unit of information. In other words, there is no 'information' without an alternative.

Intensivists face a huge number of potential alternatives. Acquisition of information reduces this number of potential states - resolving some uncertainty. Inherited genetic disease is an example where the number of alternative states is both large and, as yet, not fully defined. The On-line Medelian Inheritance in Man (OMIM) database (https://www.omim.org) as of 15th June 2019 lists more than 7,000 phenotypes and 16,092 genes. Traditional approaches of testing for a focussed panel of genes based on phenotype, requires the expertise from our clinical genetics colleagues. Their expertise is information that reduces this huge number of alternatives. However, we often do not make diagnoses in our patients despite high levels of suspicion of a genetic basis for disease.

An alternative approach, is the more 'brute force' approach to collecting information of whole genome sequencing. (3-7)

In this issue of the journal Wu et al describe such an approach to genetic diagnosis in undiagnosed critically-ill children and newborns in Taiwan. (8) They used whole exome sequencing in 40 children and newborns with suspected genetic disease to obtain 50-100,000 variants. Eleven of the 40 (27.5%) were diagnosed with previously reported mutations and another 8 had strong pointers to a new diagnosis. Mean time to a diagnosis was 6.2 days (range 4.3-9). When describing these new diagnoses, the authors state: *Many were [*the*] first example of these diseases recognized in Taiwan*.

This suggests an extraordinary powerful diagnostic technique – to identify diseases that the clinical staff may not be considering. Humans are biased by personal experience more than formal probabilities. In Bayesian terms the 'prior probability' of these diseases as assessed by the team will have been low. The post-test probability is extremely high. We rarely have the benefit of such extreme modification of the odds of a disease by a single result. The second important observation in the paper is that the information obtained by this technique often had value. The diagnostic information reduced the alternatives states so far as to prompt a change in therapy, or goals of care, in 10 cases.

Before we offer up our clinical geneticists' colleagues' salaries as possible cost-savings, we should consider the potential sources of bias in this approach. Individual patients are selected for testing. The criteria applied by Wu et al: *suspicion of a genetic condition* in critically ill children (or newborns at screening), are wide. The level of suspicion will vary

between observers based on personality and experience. This might include a judgement of how abnormal a plasma ammonia level is in a sick newborn. This judgement will change with time. The background genetic diversity of the study population will also influence performance. Taiwanese results might not be reproduced in London, Philadelphia or Cape Town.

The choice of WES – examining the coding element of the genome only – rather than its big brother: whole genome sequencing (WGS) is a potential source of bias. A recent review concluded that *"from a clinical / technical point of view, WGS is the better*[approach]" in that it provides more complete coverage of the genome. (9) Both techniques present a challenge of how to filter tens of thousands (WES) to millions of variants (WGS) down to the specific ones causing disease. This filtering cascade is subject to bias given our incomplete understanding of rare disease genetics.

Most known rare disease genes result from the identification of pathogenic coding variants. We can annotate and interpret these coding 'protein-altering' variants accurately. Analysis of large public genetic databases allows us to estimate these allele frequencies. Hence we can remove common variants since these are not compatible with the rare incidence of the disease. Combining just these two filters (protein-altering and rare frequency) removes >95% of the variants generated in most WGS studies.

Having parental data available permits further filtering on inheritance patterns. Finally, a panel is applied so that only variants within genes thought to be relevant to the phenotype are evaluated. This can be a powerful filter but also a potential major source of bias. Wu at

al describe a text-mining approach to phenotyping with few details but including putting descriptive key words into pubmed. This prone to human selection and representation bias – where features of our own mental model of a genetic condition are more likely to be searched for. A more objective approach might be the use of artificial intelligence led scanning of patient's health-data to focus on a bespoke gene panel. This method has reported some success. (10)

Even with optimal WGS filtering, diagnostic rates are typically <50%. To improve this, we will have to devise additional methods to explore our data. The ways to do this are exhaustive but include annotation of the non-coding (98% of the total) genome, consideration of polygenic causations (11) and accurate identification of complex structural variants (12).

We have never had so much information available to us. We can resolve more uncertainty than ever before to benefit of many of our patients. At the same time, we are increasingly aware of how little we know; we can see a little more clearly the true scale of the residual uncertainty. Managing this remains our biggest challenge.

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