Sequencing technology for epilepsy diagnosis

ASSIGN BUSTER

Epilepsy:

It is estimated that at many as 10% of the general populace will experience a seizure in their lifetime. (Persad *et al.,* 2003) Epilepsy is a chronic neurological disease defined by its characteristic symptom, recurrent and spontaneous seizures. (Berg *et al.,* 2011) (Asher Y *et al., 2012)* Epilepsy is a common condition with an occurrence of up to 3% in the population (Deng H *et al.,* 2013). According to Pal D. k *et al., (2010)* over half of epilepsies have a genetic cause. A large body of research has gone into identifying the genetic causes and corresponding molecular mechanisms of epileptic disorders. Epilepsies have a wide variety of causes including dysfunctional ion channels (channelopathies), abnormal brain development and errors of metabolism etc. (Garofalo S et al., 2012) Research in the genetic mutations behind epilepsies is vital in further understanding the pathology of the many different conditions and developing superior treatments for the afflicted patients.

Early Onset Epileptic Encephalopathy:

Epileptic Encephalopathies are a large number of rare (prevalence of 1 <2, 000) and devastating epileptic disorders. (Hennekam R, *et al., 2010).* The symptoms of an EE generally include severe and recurring seizures along with cognitive and developmental delay and/or deterioration. This cognitive and behavioural decline may be influenced by the seizure activity as well as the underlying mechanisms of disease (Kaiman B. A. *et al., 2012).* The term Epileptic Encephalopathy encompasses a wide variety of syndromes with a diverse range of genetic causes and considerable overlap with other

syndromic disorders such as Autism and Mental Retardation. (Berg *et al., 2011).* The overlap in these syndromes and continuing progression in our understanding of EE's has resulted in

A. T. *et al* (2010) emphasising that the term epileptic encephalopathy should be viewed as a " concept and a description" of the wide spectrum of epileptic conditions with an encephalopathic course that are being observed in the clinic.

A key diagnostic feature is that Epileptic Encephalopathies tend to occur in infancy to early childhood. While adults can suffer from epilepsy with encephalopathic features this does not tend to be as severe as the Early Onest Epileptic Encephalopathies (EOEE). Children suffering from EOEE's rarely make it to adulthood due to their poor prognosis. Berg A. T. *et al* (2010). While more research is coming to light on the potential lasting damaging effects of seizures (Berg *et al., 2011)* what is certain is that the early onset and repetition of the severe seizures present in EOEE's is devastating to the early development of patients and their continued growth. As the patients progress from a neonate towards childhood their symptoms will also progress. For example seizures in neonates may not be observable to a clinician as the axonal pathways are not fully myelinated yet and prevent the " surge" from reaching the motor cortex. Once the brain is fully myelinated the seizures would become observable clinically even though the onset was much earlier in the neonatal stage. This progression of the clinical presentation of epileptic seizures is mirrored in the likelihood of infants suffering from Early Infantile Epileptic Encephalopathy (Otohara syndrome) to develop into Infantile Spasms (IS) at 3-6 months in life. Patients' suffering

from IS tend to develop into Lennox-Gastaut syndrome in childhood. (Asher Y *et al., 2012)*

Diagnosis and treatment:

As seen in table 1 and table 2 there are a number of epileptic encephalopathies with a clear enough presentation to make an accurate diagnosis based on the clinical symptoms alone. However due to the rarity of these conditions and the occurrence of EOEE's with unclear presentations (I need a %?) confirmation of a candidate mutation (gene?) is considered the gold standard when it comes to EOEE diagnosis. (Lemke J. R. *et al* ., 2012) In Lemke J. R. (2012) it was shown that when comparing the diagnostic results of a target gene panel against a clinician's suggested diagnosis that the gene panel results matched the suggest diagnosis in the EOEE's with clear presentations. This accounted for 50% of the cohort. However for the remainder of the cohort with unclear EE symptoms there very few suggested diagnoses and not all of these were accurate. It has been suggested by Kay C, (2012) that the significant failure to confirm a genetic diagnosis for unclear EE's is due to the role of de novo variants as candidates. While we may have the above lists of confirmed disease and causative gene mutations in tables 1 and 2 there are still many other EOEE's without validated genetic causes that could be the result of de novo mutations in the patients. Considering the ever increasing list of epilepsy linked (but not validated) genes, 265 of which identified in (Lemke J. R. *et al.,* 2012)) it is clear that the current gold standard of diagnosing EOEE's isn't high enough. A case study shown by Zupanc M. L., (2009) recorded the diagnostic process of a patient " Kay" that originally presented with slight head drops at 7 months old.

Correlating the head drops to an epileptiform EEG (in this case a “ generalised high-amplitude burst of polyspike, spike and slow wave discharges”) allowed certain conditions to be ruled out however it was highlighted that while a confident diagnosis was reached there were alternatives that couldn’t be ruled out. Difficulty in EOEE diagnosis can stem from the multitude of potential genetic causes available for some presentations which can lead to a clinician wandering blindly picking candidates for Sanger sequencing to identify a candidate mutation. (Lemke J. R. *et al.,* 2012)

Next generation sequencing technology:

If the issue in diagnosing EOEE’s is the lack of validated genetic causes for the different presentations then the most comprehensive way to investigate the matter is to examine the EOEE genomes in comparison to controls and identify the candidate genes. Next generation sequencing technology has provided the means to do this and recent years have seen a surge of NGS based studies in EOEEs and their genetic causes. (Lemke *et al.,* 2012) (Veeramah K. R. *et al.,* 2012) (Veeramah K. R. *et al,.* 2013)

The original human genome project was officially announced as completed in April 2003 (Wheeler D. A., *et al.* 2013). This project made use of the hierarchal shotgun sequencing method (Chial H *et al., 2008).* This entailed the use of bacterial artificial chromosome clones (BAC) which each housed a 100Kb fragment of DNA. Over 20, 000 over these BAC clones were mapped to the human genome and the order in which these BAC clones would be aligned was the tiling path that would be followed to sequence each human

chromosome. The BAC clones are further sub divided into 2 Kb fragments (appropriately sized for sequencing) and these are sub-cloned into plasmid vectors and the fragments that will undergo sequencing. The sequencing was undergone by capillary electrophoresis methods. As long as there is sufficient overlap between these sequences they can be aligned to recreate the BAC clone structure. Then using the BAC clone mapped tiling path the sequences of the BAC clones can be aligned to create a contiguous stretch of sequence that represents the human chromosome. (Mardis E. R., 2008)

The advances made in this area to create the next generation sequencing technologies involve a movement away from capillary electrophoresis methods and changes in the methods of how the DNA fragments are assembled to produces the genome sequence. Whole Genome Sequencing removes the use of BAC clones and instead the genome is fragmented into different distinct size classes and placed into plasmid and fosmid subclones. By generating paired end reads and using the number of bases between these reads based on the size classes the genomes can be sequenced quicker than using BAC clones. Next generation sequencing platforms allow for massively parallel DNA sequencing and come in a variety of different set ups. As the need for BAC clones has been removed the sample preparation process is much quicker and cheaper than the method used in the original human genome project. Also while the actual run time of the next generation sequencers is longer than that of the capillary based platform the final yield of reads is much higher (from 96 reads on the capillary platform to up to tens of millions on a massively parallel system). This explosion in genomic sequencing technology less than two years after the completion of the first

human genome project has provided an astonishing change in the pace of genomic research. (Mardis E. R., 2008)

NGS read diagrams?

Next generation sequencing and epilepsy:

The study of genetic diseases including the EOEE has benefitted greatly from the genome sequencing revolution. Whole genome sequencing techniques have been used to identify a de novo variant in the SCN8A gene which encodes voltage gated sodium channel pore-forming alpha-subunits. Following discovery of the variant the effects of the variant on channel function was observed and shown to implicate the gene's involvement in EOEE and Sudden Unexpected Death in Epilepsy (SUDEP). (Veeramah K. R., *et al.,* 2012). Targeted gene panels (next generation sequencing of a list of target genes coding regions only) has proven effective in diagnosing clear presenting EOEE and even providing a potential causative variant when no clinical diagnosis is possible. Most impressive of all was the use of whole exome sequencing (WES) to identify de novo candidate variants of relevance in 7 out of 10 children and potentially identifying 3 new genes that could be linked to EOEE (Veeramah K. R. *et al.,* 2013)

WES is the process of sequencing only the human exome rather than the whole genome. The exome consists of all the coding regions (exons) of the genome. Even though the exome only accounts for 1% of the whole genome it is estimated to contain 85% of disease causing mutations (Choi M *et al.,* 2009). As of April 2013 U. S. National Human Genome Research Institute claims to sequence a whole genome at the price of $5826. The Howard

Hughes Medical Institute claims to sequence the whole exome at the all-inclusive cost of $500. The Broad Institute of Massachusetts Institute of Technology estimates that whole exomes can be sequenced at four times the rate of the genome in their facility (Perkel J. M 2013). This rapid and ongoing reduction in cost is mirrored at the rate WES projects are being undertaken. With a date filter for the end of 2010 on Pubmed I performed the search “ exome sequencing” and found a total of 44 articles. By the end of 2012 this 805 and by the end of 2013 the number had more than doubled to 1, 751.

Whole exome sequencing projects have the advantage of not only being cheaper and faster than whole genome projects but also are a more comprehensive option than targeted gene panels for identifying EOEE candidate variants. While not as complete in whole genome sequencing in scope 85% covers a significant amount of data to be analysed and only leaves a 15% chance of not finding a candidate variant. It should also be noted that the exome is much easier to process and analyse while the whole genome includes non-coding areas which we are currently not really able to analyse in such a way to validate the relevant link between disease and non-coding variants (Perkel J. M 2013).

Hypothesis:

The question we are trying to answer with these projects is “ Can whole exome sequencing detect candidate disease causing variations in early onset epileptic encephalopathy patients”? As this project involves isolated probands and one family trio it also raises the question of whether the

inclusion of parent WES data allows for more rapid and accurate variant analysis. As such the end research goals are to identify likely and relevant disease candidate variations in the proband WES data and confirm their presence with Sanger sequencing

The benefits of WES studies in EOEE candidate variants are numerous and important. Identifying a confirmed candidate mutation can allow an affect family to receive genetic counselling. If a clear mechanism of disease can be ascertained from the disease candidate then potentially alternative anti-epileptic therapy can be implemented based on the specific condition to improve patient prognosis even slightly. Along with this as EOEE have such a wide variety in causes and mechanisms some can respond poorly to some standard anti-epileptic drugs and lead to rapid deterioration of the patient. Identifying a genetic cause can prevent this from happening. (FIND REFERENCE) On a larger scale understanding the complex mechanisms of these devastating disorders is the only way to improve and develop treatments for these conditions and improve patient prognosis. Understanding how these rare forms of epilepsy manifest may in turn provide clues into the mechanisms of the more common forms of epilepsy. (FIND REFERENCE) As more research is beginning to highlight the role of earlier onset of seizures on the developing brain and the encephalopathic effects WES could become an effective screening tool in the future that would allow early intervention to control or prevent these devastating seizures and greatly improve patient standard of life and change EOEE to a more manageable condition. (Berg *et al.,* 2011)

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