Development of accurate diagnosis and treatments for HNF1A-MODY

The importance of distinguishing monogenic Mature-onset Diabetes of the Young (MODY) from polygenic forms of Diabetes

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Abstract

Diabetes is one of the major global health problems facing modern society but is poorly defined and subtypes are commonly misdiagnosed due to the various complex pathways that can if defective lead to hyperglycaemia. The distinction between defective pathways causing hyperglycaemia is critical for correct diagnosis and the development of adequate target treatments. Mature-onset diabetes of the young (MODY), a monogenic subtype has only been genetically distinguished from polygenic forms in the past 20 years however clinical diagnosis has not reflected this current knowledge and requires improvement. Diagnosis of HNF1A-MODY in particular, the most common form of MODY, has a substantial impact on patient quality of life as treatment with low dose sulfylnureas are more effective than insulin due to the nature of the defective pathway. This treatment is not a long lasting option and does not prevent development of disease progression however with the knowledge of HNF1A-MODY defective pathways targets for increasing beta cell mass have been identified which can pave the way for treatments to slow disease progression.

The importance of distinguishing monogenic Mature-onset Diabetes of the Young (MODY) from polygenic forms of Diabetes

Diabetes is one of the major global health problems facing modern society (Ashcroft & Rorsman, 2012). All forms of diabetes result in impairment to insulin production, action or response in the pancreatic beta cell leading to hyperglycaemia (Ashcroft & Rorsman, 2012). Hyperglycaemia was the cause of death in an estimated 3.4 million people in 2004 (WHO, 2009). and the World Health Organisation projects that by 2030 diabetes will be the 7th leading cause of death (WHO, 2011) highlighting diabetes as a case for action. There are many pathways in the pancreatic beta cells that if impaired lead to hyperglycaemia. As scientific research into diabetes develops more subtypes are being defined on these pathways and previous diagnoses are being re-evaluated (Gardener & Tai 2012).

Mature-onset diabetes of the young (MODY) is an autosomally inherited monogenic subtype of diabetes that accounts for approximately 1-2% of diabetes cases (Carroll & Murphy, 2013; Galan, et al. 2011) and was first identified as a separate subclass in the 1960s (Fajans & Bell, 2011). MODY only began to be genetically distinguished from polygenic type 1 and type 2 diabetes mellitus (T1DM and T2DM) in the mid 1990s (Fajans & Bell, 2011) and 11 subtypes of MODY have now been genetically defined on the Online Mendelian Inheritance in Man, characterised their different single gene mutations (*Maturity-Onset Diabetes of the Young, Type 3; MODY3,* 2014; Vaxillaire & Froguel 2008). It is then understandable that a 2007 study into the accuracy of MODY diagnosis found that MODY was misdiagnosed in 90% of cases (Gilliam et al, 2007). This lack of identification is compounded by the reticence to perform expensive genetic testing and insufficient incorporation of newly identified monogenic forms of diabetes into clinical diagnostic frameworks (Shepard, 2009). Misdiagnosis impacts the quality of life of patients due to an inadequate understanding of their condition and administration of unsuitable treatments.

Understanding the defective pathways leading to hyperglycaemia in the various forms of diabetes is important in understanding the progression of this disease, targeting the most effective areas for treatment and developing more accurate diagnostic frameworks. The defective

gene (monogenic) or genes (polygenic) in these pathways can be mutated in various ways and can cause changes in the genetic regulation and biochemical makeup of the body (Strimbu, & Tavel, 2011). These genetic mutations are also inherited or acquired in different ways leading to various patterns of the disease within families (Galan, et al. 2011). Correct diagnosis relies on analysing these factors from the top down, beginning with the clinical factors and inheritance patterns proceeded by analysis of markers of biochemical abnormalities that can narrow down for screening for specific genetic mutations.

This review will look at the current knowledge on the HNF1A mutations and discuss the importance and deficiencies of accurately diagnosing HNF1A-MODY from other diabetic subtypes and the implications for the development of treatments.

What is HNF1A-MODY?

HNF1A-MODY, formerly known as MODY3, is the most common of the MODY with heterogeneous mutations in the *HNF1A* gene located on chromosome 12 (Colough, et al. 2014). The *HNF1A* gene encodes for the homeodomain transcription factor, HNF1-α, which is involved in the regulation and expression of many genes in the liver, pancreas and kidney (Galan, et al. 2011). Over 200 mutations in the HNF1A gene have been identified with varying effects on transcriptional activity and DNA binding affinity of the HNF1-α protein (BooB-Bavnbek, et al. (ed), 2011; Uchizono, et al. 2009). HNF1A-MODY often arises through haploinsufficiency where one functional copy of the *HNF1A* gene does not produce enough HNF1-α protein, dominant negative forms are also found where both *HNF1A* are mutated and neither produce functional HNF1-a (Colough, et al. 2014). Some tissues expressing HNF1-α mutations don't appear to have any clinical symptoms indicating that one functional wild type allele may be sufficient in some cell types (Galan, et al. 2011). The specific phenotype of HNF1A-MODY is dependent on differences in the nature of the mutation and the characteristics of the specific target gene. The full effect of a dysfunctional HNF1-α is yet to be understood due to the diversity of the target genes (Galan, et al. 2011) however the common phenotypic symptoms include progressive beta cell loss leading to hyperglycaemia, low renal glucose uptake and commonly no association with pancreatic autoantibodies, obesity or metabolic syndrome (Murphy, Ellard & Hattersley, 2008).

Developing a diagnostic framework for genetic testing

The genetic distinction of MODY from T1DM and T2DM only began in the mid 1990's (Fajans & Bell, 2011). Previous diagnostic frameworks of diabetes have been limited by their only recognising two categories of diabetes, T1DM and T2DM with very little overlap (Dabelea, et al. 2011). Even after the causative mutated genes of some MODY subclasses were identified in the mid 1990's (Fajans & Bell, 2011) the physiological framework proposed by the American Diabetes Association (ADA) in 1997 only focused on the two main factors two distinguish diabetic subclasses: insulin deficiency due to autoimmune destruction of beta cells (T1DM) or insulin restistance with some insulin deficiency (T2DM) (Dabelea, et al. 2011). As the SEARCH for Diabetes in Youth study demonstrated there is a spectrum in diabetes and a range of mutations and defective pathways that can lead to similar symptoms of hyperglycaemia of varying severity (Dabelea, et al. 2011). Therefore diagnostic frameworks need to reflect this to enable accurate diagnosis. The cost of genetic testing is expensive and is often avoided, developing these diagnostic frameworks will help narrow down individuals that require further genetic testing and improve the cost effectiveness of genetic testing (Carroll & Murphy, 2013). Carrol & Murphy (2013) have proposed one of the most developed diagnostic algorithms to date which takes into account "atypical" T1DM and T2DM cases as well as the various MODY classes and identifies the clinical and biochemical markers required to narrow down genes that should be tested for mutations, however there are still deficiencies in the reliability of the mentioned diagnostic markers and the transition of these frameworks into clinical practice.

Multiple recent discoveries of HNF1A mutations in patients without HNF1A-MODY but increased susceptibility to T2DM (Vaxillaire & Froguel, 2008; Holmkvist, et al. 2008; Ley et al. 2011) indicates only certain mutations in HNF1A lead to the development of high penetrance HNF1A-MODY. This further complicates the accuracy of diagnosis highlighting the need for further research is required into phenotypic effect of the type of location of the mutations in *HNF1A*. Without this knowledge, the diagnostic use of genetic testing is compromised even in combination with other diagnostic markers.

Age of onset no longer a reliable diagnostic criteria

The variation in the age of onset is dependent on various combining factors and should no longer be regarded as clear and reliable diagnostic criteria for MODY or other diabetic subtypes (Fajan & Bell, 2011). Initially age of diagnosis was used as a major distinguisher between diabetic subtypes with T1DM being previously termed juvenile-onset due to the common young age of onset due to a genetic defect manifesting early in life and T2DM previously called maturity onset, believed to arise from poor diet and weight management over life time (Fajans & Bell, 2011). As a result of these definitions any diabetic cases that arose in youth was classified as T1DM and T2DM in adulthood (Dabela, et al. 2011). Mature onset diabetes of the young (MODY) was therefore named as such due to the early age of onset but with some symptoms of T2DM (Fajans & Bell, 2011). However the age of onset is not clear-cut for each subclass due to multiple interacting factors, diminishing its ability to be used as a distinguishing diagnostic factor. Recent analysis of HNF1A onset ages illustrated that only 63% of patients carrying HNF1A mutations develop symptoms by 25 years of age, 79% by 35 years and 96% by 55 years (Murphy, Ellard & Hattersley, 2008).

A genome wide association test to identify genetic factors that altered the age of onset was unable to find a specific gene responsible for the variation (Allen, 2010). This indicates a complex relationship of genetic and environmental factors (Buchbinder, et al. 2011; Allen, 2010). Individuals are likely to develop symptoms at a younger age when they are heterozygous compared to homozygous for HNF1A mutations due to gene dosage effects (Ashcroft & Rorsman, 2012) or when a missense mutation is found in the first six exons compared to missense mutations in the terminal exons 8-10 or the transactivation domain (Colclough, at al, 2014; Murphy, Ellard & Hattersley, 2008). Additionally the presence of polygenic T2D variants in combination with *HNF1A* mutations causing HNF1A-MODY have been found to reduce the age of diagnosis, independent of the *HNF1A* mutation location and other genetic and environmental factors (Allen, 2001). Not only does the presence of polygenic diabetes risk variant genes decrease the age of onset in patients with HNF1A-MODY but the presence of polygenic diabetes in the parental genome is also believed to influence the phenotype of HNF1A-MODY (Allen, 2001). Studies on the family history and pedigree of diabetes found that HNF1A-MODY patients generally developed symptoms at a younger age and greater severity when the parent without

HNF1A-MODY suffered from T2D (Allen, 2001). Furthermore studies have linked intrauterine exposure to hyperglycemia to reduce age of onset by ~ 12 yrs (Stride, et al 2002). These results indicate that although HNF1A-MODY is an autosomal dominant monogenic disease, the phenotype of the disease is also influenced by other genes and external factors such as the interuterine environment during development and paternal diabetic status. There is discussion however that decreases in age of onset of HNF1A-MODY in patients with a family history of polygenic diabetes could be due to increased family awareness and testing that the diagnosis is earlier rather than the onset (Stride, et al 2002; Allen, 2010).

Defective pathways leading to hyperglycaemia in HNF1A-MODY

The distinction between the causative pathways of hyperglycaemia between diabetic types is important for diagnosis in order to develop the right treatments. When diabetes was previously defined in the two broad categories, T1DM was known as insulin dependent/sensitive caused by an autoimmune destruction of the beta cells and T2DM as non-insulin dependent caused by a resistance of the beta cells to produce insulin (Dabelea, et al. 2011).. This distinction has become inadequate as a result of the spectrum of hyperglycaemia in diabetic patients and the increasing clinical trend to administer insulin treatment without a proper diagnosis of the cause of the hyperglycaemia (Dabelea, et al. 2011). Furthermore in the SEARCH for Diabetes in Youth Study which used autoimmunity and insulin sensitivity as the main etiological markers four categories were defined along this spectrum: autoimmune plus insulin sensitivity (IS), autoimmune plus insulin resistant (IR), nonautoimmune plus IS and nonautoimmune plus IR (Dabelea, et al. 2011).

Initially as beta cell mass begins depleting in HNF1A-MODY, basal insulin secretion is maintained but is not adequately increased in the presence of hyperglycaemia which often on the surface presents itself as type 2 diabetes (Allen, 2010). However in cases of severe and rapid beta cell loss symptoms may appear more like T1DM but will not show markers of autoimmune destruction (Allen, 2010). With the distinction of the spectrum categories from the SEARCH for Diabetes in Youth Study, MODY falls within the category of nonautoimmune plus IS along with undetected autoimmune cases (Dabelea, et al. 2011).

Identification of the mechanism of beta cell loss in HNF1A-MODY causing hyperglycaemia has helped to pave the way for identifying diagnostic markers and potential future methods of treatment through increasing beta cell mass. A recent study by Kirkpatrick (2011) found many genetic mutations in HNF1A lead to lower biochemical HNF1- α levels causing to a down regulation of genes including the X-box-binding Protein 1 (XBP1). A down-regulation of XBP1 compromises the functions of the ER and insulin folding machinery in the β-cell leading to apoptosis (Kirkpatrick, 2011). Progressive decrease in β-cell mass reduces insulin secretion resulting in hyperglycaemia (Galan, et al 2011). Understanding this mechanism provides avenues for further research into the use of XBP1 levels to diagnose a reduction in HNF1A expression as well as possible artificial expression systems of XBP1 to maintain beta cell mass in the absence of sufficient HNF1-α levels. A high throughput screen has also identified other genes down-regulated in the absence of sufficient HNF1- α levels which also have the potential to increase in vivo β-cell mass through maintenance and proliferation when artificially expressed (Karadimos 2012). One such identified gene, TMEM27 successfully increased β -cell mass in vivo (Karadimos, 2012; Akpinar, et al, 2005). Further testing is required into the combined therapy of XBP1 and TMEM27 up-regulation and other molecules in the prevention of HNF1A-MODY progression through increasing β-cell mass to combat the progressive hyperglycaemia. An emerging area of research has identified miRNAs, as a potential option in HNF1A-MODY for increasing β-cell mass (Plaisance, et al 2014). miRNAs are important regulators of β-cell development and function and can have suppression or activation capabilities. Specific miRNAs have been associated with the increased β-cell mass that acts as a compensatory mechanism during pregnancy or in obese patients (Plaisance, et al 2014). This could provide a potential area of research to develop miRNA therapy to restore β-cell mass and function. The effects of these miRNAs on other areas of the body need to be evaluated before the safety of this kind of treatment can be determined.

The difficulty in identifying effective biomarkers

Biomarkers are naturally occurring molecules, genes or characteristic that are quantitatively altered in response to these defective pathways and can be measured providing information for diagnosis (Strimbu & Tavel, 2011). The difficulty of finding reliable biomarkers is

obtaining one with sensitivity, specificity and defined, measureable clinical levels to accurately distinguish the associated condition (Strimbu & Tavel, 2011). Due to the broad range of genes and functions of HNF1A and the complexity of polygenic forms of diabetes, finding sensitive and specific diagnostic markers for a reliable diagnosis is difficult (Owen, 2010; Murphy, Ellard & Hattersley, 2008).

Genome Wide Association Studies (GWAS) have identified high sensitivity c-reactive protein (hsCRP) as a potential biomarker as it has a HNF1A binding site and is down-regulated in the absence of HNF1- α (Owen, 2010). This biomarker is useful for distinguishing HNF1A-MODY and HNF4A-MODY which has similar beta cell phenotypes but as CRP does not have a HNF4- α binding site its transcriptional regulation is not effected by a HNF4- α deficiency (Carroll & Murphy, 2013; McDonald, 2011). There are limitations in the use of this biomarker due to concurrent inflammation and infections leading to false positives (Bonner, 2013), therefore the use of hsCRP is more accurate at discounting HNF1A-MODY than diagnosing it (McDonald, 2011). Cystain C has also been identified as a potential biomarker of a decrease in glomular filtration rate due to the downregulation of SGLT2 in renal tubules in HNF1A-MODY patients (Nowak, et al 2013; (Ryffel, 2001).). This biomarker was suggested for use in combination with hsCRP but further analysis is required to determine how valuable the use of this biomarker is in diagnosis (Nowak, et al 2013) as low renal glucose threshold can also be confirmed by the analysis of 1,5-anhydroglucitol plasma levels (Murphy, et al 2008).

One of the most successful recently investigated biomarkers is the alteration in circulating serum miRNA levels. Serum levels of miR-103 and miR-224 have been found to be increased in INS-1 cells with a common HNF1A-MODY mutation compared to wild type INS-1 cells under the same glucose concentration. The use of these miRNAs to screen for HNF1A-MODY has proved to be both sensitive and specific (Bonner, et al 2013) and should be a priority for further investigation as a clinical biomarker for HNF1A-MODY and other MODY subtypes.

The impact of diagnosis on treatment

HNF1A-MODY patients have been found to have much better glycaemic control with low dose sulfonylureas compared to insulin or metformin (Gardener & Tail 2012; Shepard, et al 2013; Owen, 2010; Fajans, & Brown, 1993; Pearson, et al 2003). Sulfonylureas bypass glucose

metabolism and act down stream of many HNF1A targets on the K_{ATP} channel to stimulate β-cell insulin release (Murphy, Ellard & Hattersley, 2008; BooB-Bavnbek, et al (ed), 2011) Low levels of sulfonylureas are administered to avoid overloading the ER and insulin folding machinery leading to further β-cell apoptosis (Kwon, et al. 2013). Insulin is still currently the medication of choice during pregnancy due to the requirement of further research into abilities of sulfonylureas to cross the placenta and the impacts on fetal development (Murphy, Ellard & Hattersley, 2008). Dipeptifyl peptidase-IV inhibitors (DPP-IV) are also being studied for possible use in combined therapy in HNF1A MODY as they lengthen the amount of incretins circulating in the plasma in fasting conditions to improve glycemic control. (Katra, et al. 2010) Due to the progressive deterioration of the β-cells in HNF1A MODY patients generally end up on insulin when there are not enough β-cells and glucose induced to produce the required amount of insulin (Shepard, et al. 2003; Wedrychowicz, at al 2014). This supports the need for developing treatments increasing β -cell mass and highlights the importance of accurate diagnosis.

Can HNF1A be upregulated itself to restore function?

Embryonic development is generally is normal in HNF1A-MODY patients (Nagaoak & Duncan, 2010) and HNF1-α gene targets that have not been exposed to HNF1- α during embryonic development can successfully be activated postnatally suggesting that restoring β-cell function and proliferation could be achieved through genetic manipulation of HNF1A expression (Luco, et al. 2006). The success of artificial expression systems relies on diagnosis of HNF1A-MODY patients and an in depth understanding of the mechanisms and full range of gene dosage effects of HNF1- α expression throughout the body. A study on mice found that HNF1A overexpression was even more detrimental to β-cells than underexpression (Luco, et al. 2006). Overexpression was observed to inhibit the beta cell cycle activity through increased quantities of activated caspase 3 leading to apoptosis in mice (Luco, et al. 2006). The limitation of these findings are that mice models do not have defect symptoms in heterozygous states unlike humans who exhibit haploinsuffiency due to an autosomal dominant inheritance (Servitja, 2009). This indicates different genetic inheritance mechanisms of HNF1A and highlights the deficiencies in the use of mice model for HNF1A –MODY research. Although further research is required into the effects of HNF1A expression timing and quantity in human systems the results found in this

experiment highlight the difficulty in maintaining the adequate balance of protein levels which makes artificial expression systems of HNF1A difficult to manage and may not effectively replicate physiological function (Luco, et al. 2006). The recent ability to produce human induced pluripotent stem cells from cells of MODY patients opens up the opportunities for developing models to investigate the molecular mechanism underlying the effect of HNF1A mutations on pancreatic beta cell development could eventually lead to the ability to derive mature β-cells from hPSCs for β-cells replacement therapy (Teo, 2013).

Conclusions

As research continues to piece together the regulatory networks and mechanisms that can lead to hyperglycemia new subtypes of diabetes are being distinguished. The clinical diagnosis between the various forms has previously been inadequate due to a deficiency in practitioner knowledge, the lack of a clear diagnostic framework and the difficulty in identifying efficient biomarkers. As discussed in this paper, correct diagnosis is important to administer the most successful treatment and management plans to increase quality of life. Additionally distinguishing the different forms of diabetes aids in further studies of the different genetic and biochemical pathways affected leading to an increased understanding of the normal function of the pathways in the human body. The knowledge and distinction between different types of diabetes is progressing with the current research in the field, which could lead to further development of preventative and curative treatments. MODY, especially the most common HNF1A-MODY, should be suspected in any diabetic case with a family history of diabetes until MODY can be discounted by a combination of the diagnostic factors discussed above. Further research is required in the area of increasing β-cell mass to slow the development of hyperglycemia in HNF1A-MODY. Until β-cell replacement, proliferation or regeneration therapy is developed as a safe clinical treatment for HNF1A-MODY, patients rely on sulfonylureas and insulin to maintain their glycemic levels however these both have limitations and do not prevent the progression of the disease. The success of these kinds of preventative or management treatments relies on accurate early identification and diagnosis. When faced with hyperglycemic patients, practitioners should explore the possibility of currently defined subtypes of diabetes.

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