PERSPECTIVE

Recent advances and future directions for the pharmacogenetic basis of anti-VEGF treatment response in neovascular age-related macular degeneration

Age related macular degeneration (AMD) is a complex progressive neurodegenerative disease causing blindness in 30–35 million people worldwide. It affects the macula region of the retina leading to severe vision loss and legal blindness in individuals > 50 years of age (Wong et al., 2014). The precise aetiology of AMD is unknown but smoking, age and genetic factors are major risk factors for AMD predisposition (Ding et al., 2009). The genetic basis of AMD is well described with a recent study from the International AMD gene consortium (IAMDGC) reporting 52 genetic variants across 34 loci associated with the risk of AMD pathogenesis and explaining more than 50% of the genetic heritability of the disease (Fritsche et al., 2016).

The late stages of AMD, leading to severe vision loss and legal blindness are represented by either geographic atrophy (GA) or neovascular AMD (nAMD) also known as choroidal neovascularization (CNV) (Ding et al., 2009). The latter resulting in 90% of the vision loss attributed to the disease. In nAMD, the uncontrolled expression of proangiogenic molecules known as vascular endothelial growth factor (VEGF) initiates the growth of new and abnormal blood vessels which leak and cause permanent scars in the central retina.

The current treatment for nAMD is through the use of anti-VEGF agents that includes ranibizumab (Lucentis®; Roche Ltd., Basel, Switzerland; Novartis Pharma AG, Basel, Switzerland), Bevacizumab (Avastin®; Roche Ltd., Basel, Switzerland) typically used as an off-label drug to treat nAMD and aflibercept (Eylea®; Regeneron Pharmaceuticals, NY, USA; Bayer Pharma AG, Berlin, Germany) (Villegas et al., 2016). These anti-VEGF therapies have been particularly useful at treating nAMD. However, variation in patient response to the anti-VEGF treatment has been observed in clinical trials and retrospective studies. Approximately 10–15% of the patients do not respond to therapy and lose > 15 EDTRS letters VA (reviewed in Tsilimbaris et al., 2016). Currently, these patients undergo more aggressive treatment strategies or switching regimes between drugs. Identification of factors that might influence this response are therefore important to identify.

The reasons for a variable response to anti-VEGF treatment relate to either clinical and/or genetic factors. The former includes presenting baseline visual acuity (VA) associated with a so called "ceiling effect" impacting on change in VA after 3 and 12 months of anti-VEGF treatment. However, presence of intra retinal fluid (IRF) at baseline has also been shown to be associated with worse VA response after 12 months of anti-VEGF treatment (reviewed in Tsilimbaris et al., 2016). As far as genetic factors are concerned, previous studies have used candidate gene approaches to investigate their response to anti-VEGF treatment. Investigated genetic factors include the known AMD risk and angiogenesis pathway genes of *VEGF*, *VEGFR2*, *CFH*, *ARMS2/HTRA1*, *APOE*, *HIF2A*(*EPAS1*) and *NRP1* genes (reviewed in Fauser and Lambrou, 2015). However, results from these genetic association studies have mainly been inconclusive, for instance a study from Europe reported association of two intronic variants rs6828477 and rs4576072 of the VEGFR2 gene with improved vision (gain of 15 EDTRS letters) after 1 year of ranibizumab treatment in nAMD patients. In contrast, the largest pharmacogenetics study from the CATT and IVAN trials found no association of these variants with anti-VEGF treatment response (Hagstrom et al., 2013; Fauser and Lambrou, 2015).

Another approach to identify the genetic determinants of anti-VEGF response is to interrogate the genome in a hypothesis free manner using high throughput approaches such as genome wide association studies (GWAS) or next generation sequencing. Recently, our team successfully applied the approach of using a pooled DNA GWAS with findings being replicated by international partners. This identified the gene variant rs4910623 in the promoter region of the *Olfactory Receptor Family 52 Subfamily B Member 4* (*OR52B4*) gene as associated with worse VA response after 3 and 6 months of anti-VEGF treatment in nAMD patients (Riaz et al., 2016). The main outcome time point was change in VA during 6 months of anti-VEGF response. We chose change in VA as a treatment outcome variable as this is a typical readout in all clinical trials so far conducted on anti-VEGF therapy for nAMD. Another complexity was deciding what change in VA threshold was appropriate to select patients responding to treatment from those patients who were deemed not to have responded. We chose a threshold of 5 ETDRS letter VA change as the treatment outcome variable as it represented one of the criteria for retreatment after the first three monthly anti-VEGF injections (Tsilimbaris et al., 2016).

The pooled DNA GWAS represents a cost effective and efficient technique to identify genetic variants across the human genome associated with specific diseases or traits. It has been successfully applied in several complex diseases such as cancer and AMD but one of the caveats is that the findings need to be technically validated on the same patient cohort that was used in the pooled DNA discovery GWAS phase (Earp et al., 2014). We followed such an approach after performing the pooled DNA GWAS on 297 patients from a Melbourne discovery cohort. A total of 44 SNPs were selected for technical replication based on change in VA at 6 months of anti-VEGF therapy in nAMD patients. These were based on three approaches i) 37 variants were selected based on the traditional approach of selecting variants with genome wide significance $P < 9 \times 10^{-8}$), ii) two variants were present in drug resistant genes with suggestive significance ($P < 5 \times 10^{-6}$) and iii) five were non-synonymous changes ($P < 1 \times 10^{4}$) (Riaz et al., 2016).

Of the 44 variants selected, the results were validated for 70% of the variants ($P < 0.05$). Three variants; rs4910623, rs323085 and rs10198937 remained associated with change in VA after multiple correction ($P < 0.05$) at the six months anti-VEGF treatment stage. Interestingly, the rs4910623 variant also exhibited association with change in VA after three months of anti-VEGF treatment ($P = 1.5 \times 10^{-3}$). Next, these samples were replicated in an independent anti-VEGF treated nAMD cohort of 366 nAMD patients from Germany, Canada and the Netherlands. The variant rs4910623 was significantly associated following meta-analysis of both the Melbourne discovery and replication cohorts with poor VA response after 3 and 6 months of treatment ($P = 1.2 \times 10^{-5}$ and $P = 9.3 \times 10^{-6}$, respectively). As expected, baseline VA in both the Melbourne discovery and replication cohorts was significantly associated with change in VA after 3 and 6 months of treatment. We therefore adjusted all analyses in our study for baseline VA in both the cohorts.

The unique aspects of our study were through the use of a pooled GWAS approach to identify a pharmacogenetic response to treatment of nAMD that could be independently replicated. Secondly, using such a hypothesis free approach, a gene variant in the *OR52B4* was identified. In addition, an additive response to change in VA could be identified for an increasing number of risk alleles. For instance, in the Melbourne discovery cohort those individuals with the homozygous G allele had a mean change of 0.8 ETDRS letters compared to those with the heterozygous G allele (average change of 6.4 letters). Whereas patients with no risk allele (AA genotype) had a gain of 10.5 ETDRS let-

ters after 6 months of treatment. A similar trend of change in VA was observed in the replication cohort. However, the limitations of the study were the lack of 12-month VA data in the replication cohort. Additionally, the replication cohort used different treatment regimens after the first three injections and the number of injections was not available after 6 months of treatment.

The finding of the *OR52B4* gene involved in anti-VEGF response raises the question of what we know about this gene in the eye. Interestingly, the online web resource "The Ocular Tissue Database" revealed that *OR52B4* gene expression is found in the human retina and choroid (https://genome.uiowa.edu/otdb/). The *OR52B4* protein is a member of the G-protein coupled receptor (GPCR) and located in the plasma membrane of the cell. The expression of the GPCR in cells and tissues are of great interest as more than 50% of the therapeutic agents on the market target GPCRs (Flower, 1999). Thus, identification of the *OR52B4* gene indicates a role for the GPCR signalling pathway in anti-VEGF treatment outcome in nAMD patients.

Our study has identified a variant involved in response to anti-VEGF treatment, it is known that AMD represents a multifactorial disease with multiple genetic factors. As such it is likely that several genetic variants are involved in treatment response. Therefore risk models encompassing a number of genetic variants of varying effect size will need to be developed. Any pharmacogenetic response should also be considered in the context that genetic variants constitute only one aspect of this response and that clinical factors also need to be considered in any risk model. It should be noted that VA represents a functional readout of treatment and it is worth noting that anatomical features such as retinal fluid clearance and change in central macular thickness (CMT) in response to treatment may also provide a valuable readout. Whether the variants involved in determining anatomical response are the same as those associated with VA is currently unknown. Other aspects to consider are that rapid changes in imaging technology through different iterations of optical coherence tomography (OCT) that have led to more defined phenotyping of retinal features and that some studies will have GWAS, whole exome or whole genome sequence data available. All of these factors will need to be considered in the design of multicentre studies as different methodological approaches will impact on inclusion criteria and hence availability of sample size imparted by treatment regimens or through the use of different technologies. Such limitations may help explain why many studies of single candidate genes appear to have provided conflicting results. Larger multicentre studies with defined protocols are therefore warranted to provide a complete picture of pharmacogenetic variants likely involved in variable treatment response.

It is clear that much more laboratory work is required to establish the biological function of genes such as *OR52B4* in angiogenesis and its effect on anti-VEGF treatment outcome. Finally, translation of findings from pharmacogenetic studies to the clinic is limited as to date, there is no process or method for the clinician to predict how an AMD patient will respond before initiation of treatment. Further clinical studies will be required to screen patients for genetic factors such as rs4910623 before the start of nAMD therapy to predict patient anti-VEGF treatment response. Such studies will eventually allow clinicians to tailor treatment strategies based on an individual's genetic profile providing for a precision medicine approach instead of a "one size fits for all" approach. It should also offer the treating physician evidence to undertake a timely switch to other anti-VEGF agents. Last but not least, is that the development of new treatment therapies has the potential to augment current anti-VEGF treatments. These would be applied to the patient on an "as need basis" depending on their existing treatment response. While pharmacogenetic studies have great potential, the limited number of successful studies reflects in part the multi-factorial nature of this response and therefore the difficulty in identifying genetic determinants. We envisage that our study will provide some important "goalposts" for consideration in the development of such future studies.

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