**Genetic susceptibility and the Brugada syndrome**

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The genetic basis of the Brugada syndrome (BrS) has attracted much interest since the first successful foray by *Chen et al* in 1999. They discovered ‘mutations’ (or ‘rare pathogenic variants’) in unrelated index patients (or probands), of the alpha subunit of the cardiac sodium channel, *SCN5A*, found on chromosome 3 and responsible for the inward sodium current (INa). These caused a loss-of-function phenotype1. Subsequently around 20% of BrS probands were shown to harbour *SCN5A* ‘mutations’2. However, linkage studies to establish unequivocal association of *SCN5A* and BrS were not performed at this time.

When large families with *SCN5A* rare variants were evaluated, many individuals harbouring *SCN5A* variants did not show the Brugada phenotype i.e. incomplete penetrance. Furthermore, some relatives without the familial *SCN5A* variant demonstrated the Brugada phenotype, often in response to provocative drug challenge3. Either the *SCN5A* variant was not causative in these families and/or there were other genetic factors to consider.

By this time, other genes had been associated with BrS, usually by demonstrating electrophysiological phenotypes in candidate genes linked to the prevailing theory of pathophysiology: an imbalance of inward and outward currents during phase 1 and phase 2 (the notch and dome) of the ventricular myocyte action potential (AP) in favour of outward currents. Canine right ventricular wedge preparations treated to enhance the transient outward (ITo) current demonstrated transmural and epicardial heterogeneity of AP duration due to variable abbreviation of the AP dome in the epicardium of the right ventricular outflow tract (RVOT) relative to the endocardium4. Investigators identified putative loss-of-function ‘mutations’ affecting the L-type calcium current (ICa-L) in patients with shorter QT intervals2, and gain-of-function variants affecting the ITo and IK-ATP currents2. These findings were often specific to families in whom they were first described and the high frequency of ICa-L associated ‘mutations’ was not replicated2. Putative ‘mutations’ in multiple genes encoding beta subunits and channel-interacting-proteins (ChIPs) that affected INa were also detected including *GPD1L* on chromosome 3, where a large pedigree showed linkage, and *SCN1B*2.

Unfortunately, many of the variants described as causing BrS in the earlier literature were often too frequent in genetic databases derived from the general population such as the Exome Sequencing Project5 to be causative of rare monogenic disease. This included the same *GDP1L* variant detected by *London et al* and the originally described variant in *SCN1B*2. Other described *SCN1B* variants were associated with conduction disease rather than BrS6. The majority of the genes associated with BrS have therefore been re-evaluated by the CLINGEN initiative and all bar *SCN5A* were deemed not to have diagnostic utility7. The remainder may have roles as low frequency susceptibility variants or modifiers although this has yet to be proven (figure 1).

So where is this missing heritability? Genome-wide association studies (GWAS) led to the identification and replication of three loci harbouring common genetic variants that associated with the BrS in Caucasians and Japanese when compared to healthy controls8. Despite the high allele frequencies of these common variants in the general population, carrying 5 to 6 risk related alleles was associated with an odds ratio of over twenty for BrS. The variants are located at the *HEY2*, *SCN5A* and *SCN10A* loci and affect INa and ion channel expression across the myocardial wall8,9. Many more loci are likely to be associated in the future such that a polygenic risk score derived from these common variants may offer potential diagnostic utility in the future (figure 1).

It is on this background that Belbachir and colleagues report a potential new susceptibility gene for BrS10. *RADD* encodes the RAD GTPAse gene and following an exome sequencing linkage study in a BrS family, the *RADD*-R211H variant emerged as the best candidate for an autosomal dominant mode of inheritance.

An impressive and substantial body of functional evaluation was then undertaken in human induced pluripotent stem cells (hIPSC) derived cardiomyocytes from 3 affected patients. These demonstrated decreased peak INa amplitude, persistent late INa, prolonged AP duration, early after depolarisations, and cytoskeletal disturbances, all of which were confirmed when control hIPSC cell lines were gene edited for the R211H variant. The reduced INa amplitude is consistent with BrS, but impairment of ICa-L characterised in the patient derived hIPSC cardiomyocyte was not confirmed in the control line. Nonetheless, this finding was consistent with previous association of RAD with the regulation of Ca-V 1.2 channel trafficking to the sarcolemma10. Early after depolarisations were unexpected and may have been due to AP duration prolongation but may also have been symptomatic of the immaturity of the hIPSC cardiomyocyte model.

Additional evidence for *RADD* playing a pathogenic role in BrS included four-fold greater RAD expression levels in the mouse RVOT compared to right and left ventricular walls. In human myocardium expression was greater in the sub-epicardium than the sub-endocardium. The finding that the R211H variant also caused persistence of the sodium current similar to that of overlap *SCN5A* disease causing Brugada and long QT syndromes2, was unexpected as this phenotype was not present in the family. The authors explain this by the relative expression of RAD in the RVOT compared to the rest of the heart, avoiding manifestation of a more extensive repolarisation abnormality. The cytoskeletal disturbances detected in both hIPSC models caused abnormalities in cellular structure that were postulated to decrease cell to cell connectivity and conduction. This may be consistent with gap junction abnormalities in the epicardial RVOT in BrS patients11.

But what are the clinical implications of these findings? Can *RADD* already be added to the list of many Brugada susceptibility genes on current diagnostic panels or should it, like the majority of non-*SCN5A* genes be treated with scepticism? There are a few factors that elevate the current study above those that have preceded it: first is the robust linkage of the R221H variant with phenotype across the pedigree including a spontaneous type one pattern in the proband; second is the convincing reproduction of the cellular phenotype of impaired INa in both patient and gene edited control hIPSC derived cardiomyocyte models; third is the localisation of expression of the gene in the epicardium and RVOT; and finally, the potential for associated structural changes localised to the same sites. These are all consistent with the potential pathogenesis of the BrS4.

Concern remains, however, over the case-control genetic study designed to reinforce a role for *RADD* in BrS. The authors show a higher frequency of rare *RADD* missense variants in an independent group of unrelated BrS probands undergoing Sanger sequencing (3/186) when compared to an in-house general population sample (2/856). This finding was of borderline statistical significance. The additional case (N=3) and control (N=3, two in one subject) variants were all ultra-rare or absent in the gnomAD database and *in-silico* tools suggested pathogenicity in most. They did not undergo functional evaluation or segregation analysis, and all would be considered variants of unknown significance. There are also methodological issues with the analysis. The control was generated from whole genome sequencing data that may have given a different yield compared to Sanger sequencing applied in the cases. Ethnicity was not corrected for in the burden analysis which may have led to biases in rare variant frequency. Members of the very same research team responsible for this fascinating study have shown that most of the non-*SCN5A* BrS associated genes are not enriched for rare variation in BrS probands compared to controls12, despite presence of persuasive functional data. The case can therefore be made that *RRAD* as a cause of BrS is a family specific finding until there is more robust validation in other patient cohorts.

Furthermore, as *RADD* rare variants in Brugada probands and the general population are of relatively similar frequencies and characteristics i.e. low signal to noise ratio, how may we readily differentiate pathogenic variants for clinical application? The degree of functional evaluation undertaken by Belbachir et al. will not be feasible for every *RADD* variant discovered in a BrS patient. For the moment, adoption of *RRAD* alongside *SCN5A* in future diagnostic panels is premature.

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**Figure legend:**

Figure 1: The genetic susceptibility to BrS: the y - axis indicates the maximum relative risk associated with a variant whilst the x- axis indicates the minor allele frequency of the associated variant detected in the general population.