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From Whole Exome Sequencing to Patient-Specific Therapy: Another Example of How Basic Research Pays Off in Patient Care

Arthur A. M. Wilde, MD, PhD; Sami Viskin, MD

In the past 20 years, molecular genetics has successfully entered the arrhythmia field.[1,2] The identification of the genetic basis of many of the arrhythmia syndromes provided us the tools for better understanding of the pathophysiological basis of these syndromes,[2] for presymptomatic testing (potentially also prenatally with the possibility of extrauterine fertilization and selection of embryos without the identified mutation) and for timely treatment of family members of a more clearly affected proband,[3,4] for gene-specific therapy[2] and, occasionally, also for new, mechanism-driven therapy.[2] For example, gene-specific treatment has become very common in the long QT syndrome.[2] Examples of truly new therapeutic modalities, envisioned only after unraveling the molecular genetic basis and the associated pathophysiological mechanism, however, are relatively scarce. A good example is the identification of flecainide as a highly effective treatment of catecholaminergic polymorphic ventricular tachyarrhythmias. Initial experimental observations that flecainide blocks ryanodine receptors (experiments that were designed after the identified role of ryanodine receptors in the pathophysiology of catecholaminergic polymorphic ventricular tachycardia)[5] led to successful treatment of seriously affected individuals,[6] paving the way for this therapy into the current guidelines (as a class IIa recommendation) within 5 years of the first description.[7]

The case published in this issue of the *Journal of the American Heart Association* is another good example of bedside-to-bench-to-bedside research, with direct impact on clinical management.[8] A 37-year-old man presented with cardiac arrest and recurrent ventricular fibrillation (VF) and was treated with an implantable cardioverter defibrillator without reaching a clear diagnosis at first. A lower-than-normal left ventricular systolic function in the initial screening is described (potentially related to the prolonged resuscitation). The baseline ECG showed an incomplete right bundle branch block with minor right precordial ST elevation (that according to the original Consensus Paper would be referred to as a type 3 Brugada pattern).[9] The presence of early repolarization in the extremity leads cannot be judged, with just the right precordial leads available, but from the rhythm strip presented in Figure C, early repolarization does not seem to be present in the inferior leads immediately prior to the onset of recurrent VF. Notably, a procainamide challenge to exclude Brugada syndrome was negative.

The patient eventually returned with repeated episodes of VF and both the site of origin and the documented mode of onset of VF, with short-coupled ventricular extrasystoles, strongly suggested a diagnosis of idiopathic VF.[10,11] Accordingly,[12] quinidine was initiated with a good therapeutic response. Unfortunately, as too often happens, quinidine had to be stopped because of gastrointestinal intolerance. He then suffered from 9 more VF episodes, terminated by appropriate shocks, including 2 very severe arrhythmic storms, within less than 2 years. All events occurred despite drug regimens that included (at different times) lower dose of quinidine, amiodarone, mexiletine, and 2 ablation attempts, 1 directed towards the initiating ectopy, which was not present during the procedure, and a second one directed to the epicardial right ventricular outflow tract area.[13] Ultimately, the young patient responded well to the combination of cilostazol and dalfampridine (and still low-dose quinidine), a rather unique and unusual combination of "antiarrhythmic" drugs.

The beauty of this study lies in the description of the reasoning behind this choice. After the initial genetic screen for the usual suspects proved negative, whole exome sequencing was performed. "Multiple non-synonymous,

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From the Heart Center, Department of Cardiology, Academic Medical Centre, Amsterdam, The Netherlands (A.A.M.W.); Princess Al-Jawhara Al-Brahim Centre of Excellence in Research of Hereditary Disorders, Jeddah, Saudi Arabia (A.A.M.W.); Department of Cardiology, Tel-Aviv Sourasky Medical Center and Sackler School of Medicine, Tel-Aviv University, Israel (S.V.).

Correspondence to: Arthur A. M. Wilde, MD, PhD, Department of Cardiology, Academic Medical Centre, University of Amsterdam, P.O. Box 22660, 1100 DD Amsterdam, The Netherlands. E-mail: a.a.wilde@amc.nl

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nonsense, splice site, and frameshift variants were identified,[8] as well as a heterozygous A>G variant on chromosome 7 (7:154379727) within a presumed non-coding region of the Kv4.3-associated subunit DPP6." Only the latter was considered of interest (we are not informed on the other variants). Notably, DPP6 modulates the Kv4.3-encoded transient outward current (I_{TO}), a contributor to cardiac repolarization. Evidence is presented that this variant might actually be located in a putative novel coding exon of DPP6 and that this exon encodes 144 residues with a termination codon (DPP6-T). Functional studies subsequently revealed increased expression of DPP6-T (the encoded protein by the new exon), and an increased amplitude of I_{TO} in a cell model not specified. Furthermore, mathematical modeling of the resulting increased I_{TO} activity predicted abbreviation of the epicardial action potential duration, leading to increased transmural inhomogeneity assumed to be arrhythmogenic. Hence, increased I_{TO} activity was considered central to the arrhythmia occurrence, and 2 I_{TO} blockers—cilostazol and dalfampridine—were therefore selected with great success for over 2 years (only 1 recurrent event). The continued low-dose quinidine is not emphasized but cannot be neglected as low-dose quinidine has been suggested to be successful in patients with Brugada syndrome.[14] Notably, dalfampridine (4-aminopyridine) has been used extensively in experimental electrophysiological models, but as far as we are aware, not as an antiarrhythmic drug in our field. Altogether this is a beautiful example of how an integrated clinical, genetic, and experimental approach, involving different teams of investigators in multiple centers, can lead to a life-saving treatment.

The evidence presented is certainly in favor of an I_{TO}-mediated disease, putatively involving the identified DPP6 variant, despite the fact that it seems to be present in 1 to 5 out of 100 000 individuals. Indeed, very similar ventricular arrhythmias were observed in a large Dutch family with idiopathic VF and similar DPP6 overexpression and apparent increased I_{TO} activity.[15,16] The actual diagnosis in the present case is, however, not clear. The authors argue that despite a negative procainamide challenge, Brugada syndrome is the most likely diagnosis. The ECGs are indeed suspicious (Figure B in particular) but, unfortunately, electrocardiographic recordings of superiorly placed leads, which increase the sensitivity for detecting Brugada syndrome, are lacking. Also, information on the circumstances leading to the numerous VF episodes, which could be very informative, is not given. Nocturnal episodes, vagal triggers, and fever are typical for Brugada syndrome.[7] Finally, the ectopic beats initiating VF events appear to originate from the inferior part of the right ventricle (Figure C, superior axis of the VF initiating beat), which is very unusual for Brugada syndrome–related ectopy. In Brugada syndrome, the ectopy virtually always originates from the right ventricular outflow tract area.[17] This type of ectopy, including the associated very short coupling interval ($\pm$240 ms; Figure B and C) is characteristic of idiopathic VF.[10] In fact, this particular site of origin is reported in $\pm$25% of idiopathic VF cases in the series reported by the Bordeaux group.[11] Moreover, the coupling interval of the initiating extrasystoles seems to be shorter in idiopathic VF compared to Brugada syndrome; in a series of 19 Brugada syndrome patients with 33 shocks, the mean coupling interval of the VF initiating ventricular extrasystoles was 388$\pm$28 ms,[18] compared to 302$\pm$52 ms for idiopathic VF.[10] Indeed, the ectopy originates form the terminal phase of the T-wave in Brugada syndrome[18] and from the top (or even slightly before the top) in idiopathic VF.[10] Finally and importantly, the very same initiating ectopy as observed in this patient was also identified in the large Dutch family with idiopathic VF reported by our group and mentioned above, putatively also related to a gain of function of DPP6.[15,16] Our own experimental data, much in agreement with our clinical data, suggested that DPP6 overexpression was somehow limited to I_{TO} in Purkinje fibers, exclusively abbreviating the Purkinje fiber action potential (leading to a "PF early repolarization syndrome").[16] This would put the arrhythmogenic substrate in the Purkinje fiber, which are abundant in the inferior part of the RV. Local ablation of early Purkinje fiber activity was effective as an antiarrhythmic therapy in the Dutch DPP6-related family,[16] as well as in other cases with idiopathic VF.[11] In the present case, it apparently was not, but that could well be related to the absence of ectopy at the time of the ablation procedure (and as indicated no actual attempt was performed).

It should also be noted that the presented hypothesis (ie, transmural heterogeneity in action potential duration) does not find its correlate in the clinical findings of the present case. This mechanism has been proposed as instrumental to the arrhythmogenic substrate in Brugada syndrome.[19] Although the mechanism is disputed,[19] in Brugada syndrome the transmural inhomogeneity is located in the right ventricular outflow tract area as is the resulting "phase 2 reentry" (with expected ectopy from the very same area). As indicated above, in the present case the ectopy does not originate in the right ventricular outflow tract area, making it unlikely that this is the imperative mechanism.

In summary, this case report is a beautiful example of how top-quality multidisciplinary research teams can join forces for the immediate benefit of patients in critical condition. We can, and should, off-line discuss the exact pathophysiological mechanism of this type of arrhythmias, in order to learn even more on these conditions. However, the patient does not really care about all these details; he or she cares about high-level clinical and basic research as performed by this group. And that is what all such patients worldwide deserve and should have access to.

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Disclosures

None.

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