Clinical and genetic spectrum of hereditary cardiac arrhythmia syndromes

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Summary

Mechanism of arrhythmias are quite complex and we are still in the mid early phase in our understanding about the intricate and complex process of arrhythmogenesis. Various cardiac ion channels are required for the generation of action potential. Proper functions of these channels and well coordinated functions of cardiac desmosomes and gap junction proteins are fundamental to undisturbed electrical activity. Interference/hindrance in the finely orchestrated mechano-electrical properties of the heart (either due to ion channel defects, or defects in desmosomes or in the gap junctions) could cause various forms of arrhythmias. As mentioned in the beginning of this thesis, principal aim was to explore the clinical and molecular pathophysiology of yet undefined/uncharacterized inherited cardiac arrhythmias. We have identified several families from various parts of the globe (Netherlands, Sudan, Saudi Arabia and Malaysia) with novel or rarely observed familial arrhythmias. We have clinically characterized the phenotypes of the affected in these families, thereafter we have performed genetic analyses in these families in an attempt to elucidate the pathophysiology of the arrhythmias. Our study explored several fundamental insights into the mechanism of arrhythmias. We also aimed at developing a new modality of treatment in drug resistant CPVT patients. Finally, our study was aimed to identify population specific mutations frequencies in cardiac desmosomal genes in native Dutch ARVD/C cohorts. Genotype-phenotype analysis was performed, which ought to develop a population specific data on desmosomal gene mutations and their effects on clinical phenotypes in the Dutch population.

Chapter-2:

We have described two families who had CPVT as a common clinical phenotype. Apart from CPVT, additional abnormalities in sino-atrial node (SAN) function and AV-nodal function, atrial fibrillation (AF) and atrial standstill (ASS) were observed. Furthermore, left ventricular dysfunction and dilatation was present in several affected individuals. We have followed up these two families for 20+ yrs period and characterised their clinical phenotypes over time. By linkage analysis, we have mapped the disease locus to a 4 cM region on chromosome 1q42-q43. Ryanodine receptor 2 gene (RYR2) and ACTN2 is located in this locus. We could not find any mutation neither in RYR2, nor in ACTN2 genes by conventional PCR-based screening. Subsequently, we have identified a complete exonic deletion (exon-3) in RYR2 gene by multiplex ligation-dependent probe amplification and long-range PCR. We have revealed that the genomic deletion occurred due to Alu repeat-mediated polymerase slippage during chromosomal replication. This is the first and only report on a large genomic deletion in RYR2, which leads to extended clinical phenotypes, e.g. SAN-
and AV-node dysfunction, AF, ASS and dilated cardiomyopathy. These features have not previously been linked to RyR2 dysfunction.

Chapter-3:
We described the discovery of a novel variant of CPVT, which we termed CPVT-3. CPVT-3 was diagnosed in four closely related patients, inherited as an autosomal recessive trait, the disease was found to be highly penetrant already in childhood. Three children with an average age of 10±2 years died suddenly during exertion, one surviving male child had his first syncope and seizures at the age of 7 years, during playing. ECG and phenotypic characteristics are reminiscent of CPVT (1) and CPVT (2), but clinical phenotypes are more malignant in presentation in three cases first presentation was sudden loss of unconsciousness followed by death. We have performed homozygosity mapping in this highly inbred family from Sudan to determine the pathogenetic locus. A novel locus for this malignant CPVT subtype mapped to chromosome 7p14-p22. Screening of the coding regions of the positional candidate genes *SP4, FKBP9, FKBP14, PDE1C, NPY, TBX20* did not reveal a mutation. Further studies are required to identify the causative gene and mutation in this novel, autosomal recessive variant of CPVT (3).

Chapter-4 to 5:
*RYR2* consists of 105 coding exons. Direct sequencing of each exon is time consuming and not economical. We developed a novel DHPLC based screening method to identify sequence aberrations. With this method added by sequence strategy we have screened 45 CPVT patients for *RYR2* mutation/s. We have reconfirmed that mutations in *RYR2* are clustered in the N- and C-terminal domains, as well as in the central domain. Mutations detected in our study are predominantly private and 28% of the mutations are located in exon 90, followed by a considerable number of mutations in exon 3 and exon 14 (24% of total mutations). Mutation in the promoter region and also large genomic rearrangements are rare in *RYR2* comprising <5% of the aberration. Mental retardation was observed as an associated phenotype in 4 probands, with mutations exclusively in the C-terminus. *RYR2* mosaicism could occasionally occur and has implications during genetic counselling.

Chapter-6:
Though, in most cases of CPVT, β-adrenergic blocker is the mainstay of therapy. But, the protective effect of β-adrenergic blocker in alleviating the symptoms are incomplete. Many continue to have symptoms and/or documented exercise-induced VTs despite β-adrenergic blockers. We presented evidence that left cardiac sympathetic denervation (LCSD), an
antifibrillatory intervention that largely prevents norepinephrine release in the heart, might reduce these adrenergically-mediated life-threatening arrhythmias. We proposed that LCSD may represent a novel and effective treatment for those young CPVT patients who are not fully protected by β-blockers.

Chapter-7:
We have performed clinical, molecular and functional investigation in a consanguineous Arabian family with repeated miscarriages at 8-10 weeks and two fetal losses at 29th weeks and 28 weeks of pregnancy, most likely due to persistent arrhythmias originating at the intrauterine stages. Our investigation showed that the severe arrhythmia started during the 2nd trimester of pregnancy, which is a novel observation. One child who survived from the fatal consequences of the intrauterine arrhythmias was born at 32nd weeks of fetal life by elective caesarean section. Immediately after birth he was found to have profound QT prolongation (QTc= 605 ms), with 2:1 (functional) AV block and severe ventricular arrhythmias. No clinical and abnormality in ECG among the parents were found.
Candidate gene mapping found the susceptible locus encompassing the \textit{KCNH2} gene. Screening of the \textit{KCNH2} gene in the homozygous locus detected a homozygous non-sense mutation Q1070X in the HERG C-terminus in the affected children. Biochemical and functional analysis of the Q1070X mutant showed that the mutant HERG though have the properties to traffic to the plasma membrane and could form functional channels, are destroyed by the Non-sense Mediated Decay (NMD) pathway before its translation. NMD leads to near absence of HERG in the homozygous Q1070X mutation carriers causing debilitating arrhythmias (already prior to birth) in the homozygous carriers and apparently without any phenotype in the heterozygous carriers.
Findings from this study stressed the fact that current methods for investigating mutant cardiac ion channels are mostly one sided. In order to explore the disease pathogenicity, functional disintegration of the mutant channel with its interacting proteins and signal pathways also must be investigated (Morten Grunnet, PhD, FHR, Editorial commentary, Heart Rhythm, 2008; 5: 562-564), which is in addition to measure the electrical properties of the mutant channels,

Chapter-8:
We have performed clinical, molecular and functional investigation in two consanguineous Arabian families with history of sudden death of several children. Parents in both families are devoid of any cardiac symptoms and also no abnormality on ECG was detected. The history included exertion induced phenotypes among all the victims, and extreme QTc prolongation (QTc= 557ms and QTc= 529 ms, respective in proband of two families). None of
the affected individuals had any other impairment. We decided to perform a homozygosity mapping around candidate genes linked to electrical instability of the heart. Locus surrounding the \textit{KCNQ1} gene was linked to both families, which led us to screen the \textit{KCNQ1} gene. We have identified a novel splice acceptor site mutation (homozygously) in intron 1 of the \textit{KCNQ1} gene (c.387 -5 T>A), in these two apparently unlinked families. RNA analysis revealed that this splice site mutation causes incomplete transcriptional aberration of the KCNQ1 gene, leaving 10\% of the normal allele transcript intact, which restored the hearing function. Our molecular and functional data provided the first evidence that residual amount (as low as 10\%) of normal KvLQT1 (coded by \textit{KCNQ1}) current could effectively maintain the hearing function but fails to maintain cardiac repolarization characteristics within normal limits.

Additionally, we have revealed four extra low frequency aberrant isoforms emphasizing the importance of intronic and other non-coding sequences in maintaining cellular homeostasis as pathologic changes in a single nucleotide can affect splicing events at distant sites. The novel \textit{KCNQ1} mutation found in this study is very likely a founder mutation in the southern province of Saudi Arabia emphasizing its screening in the LQTS population in this mountain region of Saudi Arabia.

\textbf{Chapter-9 and 10:}

In chapter-9, we have identified the genetic pathology causal to the fatal/malignant arrhythmias in the children, investigated. All the genetic mutations identified in this study are novel in origin, never described in other populations. Our study elucidated that the LQT1 causing splice site mutation c.387 -5 T>A in \textit{KCNQ1} gene and the LQT2 causing non-sense mutation c.3208 C>T (p.Q1070X) in \textit{KCNH2} gene, are very likely founder mutations in the Assir province of Saudi Arabia. Genetic and clinical findings in this study are quite intriguing for several reasons: 1) In total, we have investigated 6-families, among them 4 are homozygous/recessive carriers for the mutations and the mutations originated from an ancestral source; 2) All the detected LQTS causing mutations are novel, being reported only in these Arab families in this study; 3) Due to the homozygous mutations, clinical phenotypes are also severer in the studied families. We suggest that the genetic and phenotypic observations stems from the high rate of consanguineous marriages in this country.

In chapter-10, we have investigated two unrelated children with LQTS originated from Kelantan province, Malaysia. In one child, we have found a LQT1 causing mutation p.Ile567Thr (c.1700T>C) in the \textit{KCNQ1} gene. We consider this a recurrent site for mutation as the mutation has been reported previously in a patient from Italy. In the second patient from Malaysia with LQTS combined with 2:1 AV block, no mutation was in any of the presently known LQTS causing genes. We also could not find any genomic rearrangement
in the main LQTS causing genes. Mutation in a presently unidentified gene or a mutation in the regulatory region (including intronic) in one of the known LQTS causing genes could be causal to the LQTS phenotype in the second patient.

**Chapter-11 to 14:**
Familial Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy (ARVD/C) is predominantly an autosomal dominant inherited heart disease with variable expression and incomplete penetrance. This study aimed to evaluate the prevalence and character of mutations in ARVD/C patients in the desmosomal genes Plakophilin-2 (PKP2), Desmoglein-2 (DSG2) and Desmocollin-2 (DSC2) and to study the mutation effect on clinical presentations. PKP2 mutations were most prevalent in Task Force Criteria positive (TFC+) patients, comprising nearly half of the ARVD/C patients (~40%) and also accounted for 70% of the cases with a positive family history for ARVD/C. Mutations in DSG2 were found in only 7% of the Dutch ARVD/C index patients. Moreover, DSG2 mutations were found with a similar frequency in our patients who were TFC−. Mutation frequency is even lower in the DSC2 gene in all groups. Compound heterozygous or homozygous mutations were found in two individual cases. In one patient, mutations in both DSG2 and DSC2 have been detected. Interestingly all three patients fall in the full blown ARVD/C group. In conclusion, in our Dutch ARVD/C cohorts, we confirm that PKP2 mutations with a familial preponderance are exclusively found in TFC+ patients, whereas DSG2 and DSC2 mutations (combined) are seen in less than 10% of TFC+ and TFC− patients. Importantly, bi-allelic or digenic mutations constituted 50% of the DSG2 and DSC2 mutation positive TFC+ ARVD/C patients, suggesting a single mutation itself, either in DSG2 or DSC2, are less likely to cause a full blown phenotype. Yet the presence of a second mutation in a second gene or the same desmosomal gene/s could worsen the phenotype. Negative T waves, an indicator of cardiac repolarisation abnormality were prevalent among the mutation carriers.