Juhani Junttila

CHARACTERISTICS OF SUBJECTS WITH BRUGADA SYNDROME TYPE ELECTROCARDIOGRAM
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Academic dissertation to be presented, with the assent of the Faculty of Medicine of the University of Oulu, for public defence in Auditorium 10 of Oulu University Hospital, on April 25th, 2008, at 12 noon

OULUN YLIOPISTO, OULU 2008
Brugada syndrome is an inherited arrhythmia disorder that predisposes to sudden cardiac death. It is characterized by its distinct ECG pattern. The purpose of this thesis was to study the phenotype and genotype characteristics of subjects with Brugada syndrome type ECG.

The first study population consisted of 2479 young male Air Force applicants and 542 healthy middle-aged subjects. The 12-lead ECG was analyzed to assess the prevalence and prognosis of Brugada pattern in Finnish population. The second population consisted of 168 patients with AF. The ECGs of the patients with family history of lone AF were analysed in order to characterize the ECG features of familial AF. The third population consisted of 200 patients with Brugada syndrome and their ECGs were analyzed for detection of distinct ECG characteristics. In a substudy, the H558R variant was genotyped and the clinical presentation of this variant was evaluated. The clinical characteristics were collected of 47 patients with induced Brugada ECG during fever or medication.

The prevalence of type 2 or 3 Brugada ECG was 0.61% in the young population and 0.55% in the middle-aged Finnish population. In a retrospective analysis, none of the Brugada ECG carriers had died. In the AF study, the prevalence of type 2 or 3 Brugada ECG was significantly higher among the subjects with lone AF compared to the healthy controls (p < 0.001). Many of the Brugada ECG carriers had a family history (> 30% of first-degree relatives) of AF. In patients with Brugada syndrome, the prolonged QRS duration was associated with previous symptoms. The R allele carriers in H558R variant had a trend towards less symptoms (p = 0.067) and had less conduction disturbances in 12-lead ECG than the HH genotype carriers (p < 0.05 in all ECG analysis). Among the subjects with induced Brugada ECG, 51% exhibited arrhythmic symptoms during the medical condition that had provoked the ECG pattern.

In conclusion, type 2 and 3 Brugada ECGs were found to be benign in the Finnish population since no mortality occurred during an extensive follow-up period. On the other hand, these ECG abnormalities seem to be a marker of familial AF. Among patients with the Brugada syndrome, a prolongation of QRS is associated with prior symptoms. The variant H558R R allele seems to be a protecting genetic modulator. Induced Brugada ECG is a medical emergency since the patients are at high risk of sudden cardiac death.

**Keywords:** Atrial Fibrillation, Brugada syndrome, ECG, genetics
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Oulu, November 2007

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**Abbreviations**

<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AF</td>
<td>Atrial fibrillation</td>
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<td>ARVD</td>
<td>Arrhythmogenic right ventricular dysplasia</td>
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<td>CPVT</td>
<td>Catecholaminergic polymorphic ventricular tachycardia</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram, -phic, -phy</td>
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<td>EP test</td>
<td>Electrophysiological test</td>
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<td>HCM</td>
<td>Hypertrophic cardiomyopathy</td>
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<td>HOCM</td>
<td>Hypertrophic obstructive cardiomyopathy</td>
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<td>ICD</td>
<td>Implantable cardioverter defibrillator</td>
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<td>LQT</td>
<td>Long QT</td>
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<td>OR</td>
<td>Odds ratio</td>
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<td>PVC</td>
<td>Premature ventricular contraction</td>
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<td>ROC</td>
<td>Receiver operating characteristic</td>
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<td>SCD</td>
<td>Sudden cardiac death</td>
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<td>SNP</td>
<td>Single nucleotide polymorphism</td>
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<td>SQT</td>
<td>Short QT</td>
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<td>VF</td>
<td>Ventricular fibrillation</td>
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1 Introduction

Since the invention of the ECG in the early 1900s by Einthoven, many clinical disorders with distinct ECG features have been recognized. In the field of heritable diseases, ECG plays an important role in gathering diagnostic and prognostic evidence of arrhythmia syndromes. The first significant arrhythmia syndrome described by Jervell and Lange-Nielsen and also Romano and Ward in the 1950-60s was also characterized by an ECG abnormality; long QT interval (Jervell & Lange-Nielsen 1957, Romano et al. 1963, Ward 1964). The rapid evolvement of molecular biology and genetics during recent decades has opened up also vast research possibilities for characterising and detecting new distinct patterns to old arrhythmia syndromes. For example, the first gene linked to long QT syndrome was detected some 30 years after the entity was recognised, but with the recently discovered short QT syndrome this genetic linkage took only four years (Brugada et al. 2004, Wang et al. 1996).

Brugada syndrome is one of the arrhythmia syndrome entities. It was first described by Josep and Pedro Brugada in 1992 as an inherited arrhythmia disorder characterized by a coved type ST-segment elevation in the right precordial leads of the 12-lead ECG with a high risk of life-threatening ventricular arrhythmias and SCD (Brugada & Brugada 1992). The typical ECG pattern may be concealed in the baseline ECG, but it can be unmasked with sodium channel blockers (Brugada et al. 2000). The diagnosis of the Brugada syndrome can be made with baseline ECG or with drug induced ECG findings. A genetic mutation causing the disease was discovered in 1998 in the SCN5A gene that encodes the alpha-subunit of the cardiac sodium channel (Chen et al. 1998). However, most patients with Brugada syndrome do not carry SCN5A mutation, despite having a typical clinical presentation. The worldwide incidence of the syndrome is an estimate 5 in 10000 and it is the second leading cause of sudden death of men under 40 years in southeast Asia, but significant geographical differences has been described (Antzelevitch et al. 2005a). The syndrome is rare in the western world, but it does carry a great economical and emotional impact because it commonly affects young men who are in their most productive phase in their careers and may have small children (Antzelevitch et al. 2005a). The penetrance of the disease is heterogeneous and thus there is a clear need to develope tools for detecting patients at high risk of SCD.

Initially, the aim of this work was to determine the prevalence and prognostic significance of Brugada syndrome type ECG in Finland. After identifying a
family with type 2 Brugada ECG, without a history of SCD and a high occurrence of lone AF, we elected to study the relationship between the Brugada type ECG and lone AF. Finally, we investigated whether baseline or induced ECG pattern and genetic analysis could be used to predict SCD in the patients with Brugada syndrome. These studies were conducted in collaboration of Dr. Ramon Brugada in the Montreal Heart Institute.
2 Review of the literature

2.1 Inherited arrhythmia syndromes

Many inherited arrhythmia syndromes are defined or characterized by the specific ECG pattern related to the syndrome. In the following chapter the most common inherited cardiac disorders causing SCD are reviewed with a special emphasis on Brugada syndrome.

2.1.1 Long QT syndrome

In 1957, Jervell and Lange-Nielsen first described a family with congenital deafness, syncopal episodes and prolonged QT interval in the ECG (Jervell & Lange-Nielsen 1957). Three of the four children in the family later died suddenly, but those family members with normal QT interval and hearing led a normal life. In the 1960s, Romano et al. and Ward independently described families with a similar clinical presentation but without deafness (Romano et al. 1963, Ward 1964). Subsequently the Jervell-Lange-Nielsen syndrome was viewed as an inherited disorder with an autosomal recessive and the Romano-Ward syndrome with an autosomal dominant trait of inheritance. The incidence of the LQT syndrome has been estimated to be 1 in 10000 in the early estimations but recently the incidence have been updated to 1 in 2000 (Roden 2008, Vincent et al. 1992b). Already in 1970s autonomic nervous system bursts were described as a major factor in triggering syncope episodes in LQT subjects and beta-blockers were found to be very effective in inhibiting these symptoms (Schwartz et al. 1975, Yanowitz et al. 1966). Many of the LQT syndrome patients experienced a syncope episode while exercising, swimming or with emotional arousal (Ackerman 1998). With beta-blockers, the 10-year mortality rate was reduced from 71% to 6% (Eldar et al. 1992, Schwartz et al. 1975, Schwartz 1985, Schwartz 1997). In the present molecular genetic era, a total of ten genes and ten subtypes have been associated to this syndrome (Roden 2008). Loss-of-function mutations in genes KCNQ1 (LQT1), HERG (LQT2), KCNE1 (LQT5), KCNE2 (LQT6) and a gain-of-function mutation in SCN5A (LQT3) have been associated with the common subtypes of LQT syndrome (Abbott et al. 1999, Curran et al. 1995, Splawski et al. 1997, Wang et al. 1995, Wang et al. 1996). The mutated
allele has been described to be transmitted more often to females (Imboden et al. 2006).

The diagnosis of LQT syndrome since the beginning has been a matter of debate for there is a considerable overlap in the QT intervals of the normal population with the patients with LQT syndrome. A corrected QT interval (QTc) of ≥ 430ms yields a specificity of 86% and sensitivity of 72%, but still many of the gene mutation carriers that evoke the LQT syndrome seem to have a normal ECG (Hofman et al. 2007). Presently the diagnosis of the LQT syndrome is based on a point chart where different ECG findings are taken into account (Schwartz et al. 1993). Genetic analyses are also often used as a diagnostic tool in the LQT syndrome. To date beta-blocking drugs remain the first line of treatment in LQT syndrome to prevent **torsades de pointes** which is a characteristic arrhythmia of this syndrome (Dessertenne 1966). According to some investigators all symptomatic, or asymptomatic patients under 40 years old, should be treated with beta-blockers (Garson et al. 1993, Vincent et al. 1992a) which is understandable in view of the safety and efficacy of beta-blocker therapy in LQT syndrome. Nonetheless, beta-blockers have not been considered being successful in treating LQT3 (Schwartz et al. 2001, Shimizu & Antzelevitch 2000). Patients who have symptoms despite beta-blocker therapy may be additionally treated with pacing (Eldar et al. 1992), cardiac sympathetic denervation (Schwartz 1997) or with an implantable cardioverter-defibrillator (ICD) which is also proposed as first-line therapy after resuscitated cardiac arrest (Kron et al. 1990). Altogether, presently ICD is commonly used in case of symptom occurrence during beta-blocker therapy.

Subsequently different QT and T-wave patterns in ECG have been described to represent distinct forms of LQT syndrome. The T-wave duration is longer in LQT1, LQT2 patients usually have small or notched T-waves and in LQT3 the onset of T-wave is usually prolonged. With the help of these characteristics, in many patients the different subclass was correctly evaluated in ECG analysis (Zhang et al. 2000). In the risk stratification of symptoms (before the age of 40 years) in long QT syndrome, genetic loci for the syndrome (KCNQ1-LQT1, KCNH2 (HERG)-LQT2, SCN5A-LQT3 mutation), gender and QTc measurement have been stated to be independent predictors of risk (Priori et al. 2003, Zareba et al. 1998). Patients with LQT1 have the lowest risk according to genotype compared to LQT2 and LQT3. Furthermore, the mortality when the event occurs is the highest in LQT3 (Zareba et al. 1998). Female gender was linked to higher risk only in LQT3, and QTc ≥ 500ms was associated with higher risk only among
LQT 1 and 2 patients (Priori et al. 2003). In the total LQT population, young age is a “cumulative” risk marker since most of these events occur in younger patients (Zareba et al. 1998).

The LQT syndrome has also been extensively studied in Finland. The Finnish eccentric character in LQT syndrome is the existence of founder mutations. Due to the homogenous basis of the Finnish genetic heritage (Peltonen et al. 1999), as many as 73% of all LQT patients with the uncovered mutation have one of four founder mutations in either KCNQ1 (G589D, IVS7-2A > G) or HERG (R176W, L552S) (Fodstad et al. 2004). In addition 24h Holter recording studies in LQT patients have been successful at detecting symptom history, yielding evidence of transmural dispersion and have revealed the ambulatory effects of beta-blocker therapy by the means of T2-/T1-ratio in LQT1 and 2 patients (Viitasalo et al. 2002, Viitasalo et al. 2006 a, Viitasalo et al. 2006).

2.1.2 Short QT syndrome

A syndrome with high risk of SCD and AF and SQT interval was described in 2000 (Gussak et al. 2000). Subsequently a definite hereditary link was described with SQT and SCD (Gaita et al. 2003). The characteristic ECG pattern of the syndrome includes short QTc interval of 280-330ms and high peaked T-waves in right precordial leads. Molecular genetics have played a major part in the history of this recently discovered syndrome since already in 2004 the first gene mutation causing the syndrome was uncovered (Brugada et al. 2004). To date, three genes, i.e. KCNH2 (HERG), KCNQ1 and KCNJ2, have been linked to the syndrome which also bears resemblance to LQT syndrome (Bellocq et al. 2004, Brugada et al. 2004, Priori et al. 2005). In contrast to the LQT syndrome, in the SQT syndrome all of these genes were found to have mutation resulting in a gain-of-function of the potassium channels in the myocardial cell. Similarly to the situation with the LQT syndrome, the cut-off point of normal and SQT interval is vague. Some affected individuals in SQT families have been described to have QTc intervals of over 340ms (Maury et al. 2005), but also recent studies have shown that the prevalence of QTc under 340ms in the general population is 0.4% and none of these subjects had died during an extensive follow-up period (Anttonen et al. 2007). Similar finding has also been described in Italian population (Gallagher et al. 2006) This finding challenged the worldwide outlook of all subjects with SQT needing an ICD. Presently no diagnostic criteria for SQT syndrome have been proposed. The diagnosis is based on an evaluation of
symptoms and ECG findings; although all symptomatic patients can be considered to have been asymptomatic until the day when they experience the first symptoms and that symptom may well be SCD. Most of the families described so far have had high penetrance of the syndrome within the family and this has evoked symptoms in most cases (Giustetto et al. 2006). The only effective treatment for SQT syndrome to date is ICD therapy (Schimpf et al. 2003). Different drug therapies such as sotalol and quinidine have been examined, but no large randomized studies have been conducted yet (Brugada et al. 2005, Gaita et al. 2004). EP testing has been used as an evaluative method of risk for SCD of patients with SQT syndrome, but major issues remain to be resolved with respect to the refractory periods of the cardiac muscle. SQT syndrome patients have a naturally short refractory period in the myocardium and thus the extrasystoles in pacing have to be delivered with very short coupling intervals, less than 200ms, which may cause problems in the sensitivity of the test. Solving the dilemma of SQT syndrome diagnostics and prognostic evaluation will demand more studies in the near future.

So far, only one family with SQT syndrome has been described in Finland (Anttonen et al. 2004) and extensive studies in ECG risk stratification marker studies are ongoing in the European SQT syndrome patient registry.

2.1.3 Catecholaminergic polymorphic ventricular tachycardia

CPVT is an inherited arrhythmia syndrome that is characterized by adrenergically provoked polymorphic ventricular arrhythmias in structurally normal heart. This disorder was first described in 1975 (Reid et al. 1975) and its symptoms are usually syncopal episodes triggered by exercise or emotional stress similar to the situation in LQT syndrome (Priori et al. 2001). CPVT is considered as a very malignant disorder, even when compared to other arrhythmia syndromes, as it provokes symptoms in as many as 80% of the patients under 40 years of age. The mortality is also high, with 30-40% overall incidence by the age 40. (Mohamed et al. 2007) The genetic background for the disease was described first by a Finnish research group when they identified the locus for CPVT to 1q42-q43 (Swan et al. 1999). Subsequently in 2001, the Finnish group and an Italian group almost simultaneously discovered the first mutations in RYR2 gene (Laitinen et al. 2001, Priori et al. 2001). Major advances in diagnosis and pathogenesis have been made after the genetic background was revealed. After the discovery of the RYR2 mutation in CPVT, mutations in CASQ2 were discovered in the recessive form of
CPVT (Lahat et al. 2001). The ryanodine receptor gene mutations have been associated with the autosomal dominant form.

The clinical diagnosis of the CPVT can only be made from exercise ECG since the disorder does not exhibit any characteristic ECG marker that can be detected from baseline ECG but presently genetics is also a prominent diagnostic tool in this disorder. In the exercise ECG the most common finding in CPVT is the growing amount of polymorphic PVCs which appear with exercise and the occurrence of bidirectional VT episodes. The CPVT characteristic VT can be differentiated from *torsades de pointes* by the axis of the arrhythmia. In *torsades*, the QRS axis turns gradually and chaotically, but in CPVT bidirectional VT, the beat to beat axis rotates 180 degrees every time. During the exercise ECG, the findings appear and diminish gradually with the level of exercise. (Mohamed et al. 2007)

The first line treatment for CPVT is beta-blocker therapy similar to LQT syndrome (Sen-Chowdhry & McKenna 2006). Beta-blockers decrease and depress the adrenergic tone and thus reduce the occurrence of exercise and emotion related arrhythmias. Although, in theory the concept of beta-blockade fits perfectly with the pathogenesis of this syndrome, the efficacy of the treatment appears to be less than anticipated. The efficacy of beta-blockers is not as high as in LQT1 but rather at the level of their use in LQT2 (Priori et al. 2004). There are two major studies, in one the incidence of SCD was 10% (Leenhardt et al. 1995) and in the other study 30% of the patients required ICD therapy despite antiadrenergic medication (Priori et al. 2002b). In the ICD study described by Priori et al, 50% of the patients received appropriate shocks from the device during a two-year follow-up (Priori et al. 2002b). Nonetheless, it is recommended that all patients with ICD should be treated also with full dose beta-blocker medication as patients may die suddenly in case of an electric storm though they have been provided with an ICD (Mohamed et al. 2007).

### 2.1.4 Hypertrophic cardiomyopathy

HCM was accepted as a clinical entity in the 1950s. The definition of HCM has been determined over decades to refer to myocardial hypertrophy in the absence of hemodynamic stress that is sufficient to account for the degree of hypertrophy or histopathological findings of myocyte disarray. (Brock 1957, Hauer et al. 2001, Teare 1958) The estimated prevalence of the disease is 1 in 500 and it is the most common cause of SCD in young people. The overall annual death rate in HCM is
~1%, whereas that in patients with left ventricular outflow tract obstruction (hypertrophic obstructive cardiomyopathy, HOCM) the mortality rate is ~2%. (Codd et al. 1989, Hada et al. 1987, Lipshultz et al. 2003, Maron et al. 1995, Nugent et al. 2003) From the 1980s, there has been a growing recognition that most HCM cases are familial. A total of 10 cardiac sarcomere structural protein encoding genes have been associated with HCM (Geisterfer-Lowrance et al. 1990, Kimura et al. 1997, Mogensen et al. 1999, Niimura et al. 1998, Poetter et al. 1996, Satoh et al. 1999, Thierfelder et al. 1994). The most widely recognized gene is the β-myoglobin heavy chain where a mutation is found in 35-50% of all HCM cases (Marian & Roberts 2001). In Finland, due to the homogenous genetic background of the population, founder mutations in myosin-binding protein C, alpha-tropomyosin and beta-myosin heavy chain genes account for 61% of familial and 40% of sporadic HCM cases (Jaaskelainen et al. 2004). In addition mutations in mitochondrial DNA have been associated with HCM (Obayashi et al. 1992).

The characteristic symptoms of the disease are chest pain and dyspnea during exercise. Syncope during exercise is usually a result of HOCM but if unexplained in HCM without obstruction, it is a serious marker of SCD risk. The golden standard of diagnosis in HCM is echocardiography. Unexplained left ventricular wall or septal diameter over 15mm is sufficient to indicate the diagnosis of HCM. (Elliott & McKenna 2004, Richardson et al. 1996) An exercise test is often used also in prognostic evaluation, since most of the patients with HCM or HOCM do not display symptoms during rest (Elliott & McKenna 2004, Richardson et al. 1996). The treatment strategies for HCM vary from medication to surgery. Beta-blockers are the backbone of medication but also calcium-blockers are used in HCM patients (Maron et al. 2003). Patients who have serious symptoms related to HOCM can be treated with surgical myectomy or ethanol ablation to decrease the obstructive component of the septum (Faber et al. 1998, Morrow et al. 1975). An ICD is the only effective treatment to prevent SCD in high risk HCM patients (Maron et al. 2003). According to recent guidelines, ICD implantation is indicated in patients with documented sustained ventricular arrhythmias or aborted SCD and prophylactic ICD implantation is recommended in asymptomatic patients with a clear family history of SCD at a young age (Hauer et al. 2001). Genetic counselling is an important part of the HCM patients’ examination, since there is a strong familial background in the disease (McKenna et al. 1997). Genetic testing allows the affected family members to be discovered and treated.
The ECG characteristics for HCM are mostly due to hypertrophy rather than abnormal ion channel function which obviously is not the case in HCM. Atrial enlargement, left ventricular enlargement in precordial leads, repolarization abnormalities and Q-waves mostly seen in the inferior leads are commonly observed in HCM patients. (Fananapazir et al. 1989, Lemery et al. 1990, Maron et al. 1983, Savage et al. 1978) In addition short PR intervals and slurred QRS upstrokes, but not those changes seen in the Wolf-Parkinson-White syndrome, are more prevalent among HCM patients. (Krikler et al. 1980) The characteristic arrhythmias in HCM are premature ventricular beats, non-sustained ventricular tachycardia and supraventricular tachycardia (Elliott & McKenna 2004).

2.1.5 Arrhythmogenic right ventricular dysplasia

In 1977, the term arrhythmogenic right ventricular cardiomyopathy or arrhythmogenic right ventricular dysplasia (ARVD) was first proposed in a report describing six patients with sustained ventricular tachycardia without overt heart disease (Fontaine 1977). Early reports also described localized right ventricular involvement but now it is clear that left ventricular (LV) involvement can be present in the advanced phase of this disease (Corrado et al. 1997, Pinamonti et al. 1996, Thiene et al. 1988). The characteristic pathogenesis in the disease is fibrofatty replacement of cardiac muscle in the right ventricle (Corrado et al. 1997). The prevalence of ARVD is approximately 1 in 5000 and it accounts for 5% of SCDs in under 65 year old subjects in the US, though this might be an underestimation because of diagnostic difficulties (Norman & McKenna 1999, Peters et al. 1999, Tada et al. 1996). The estimate of the annual mortality rate due to ARVD is 3% without treatment and 1% with treatment (Aouate et al. 1993). In the 1980s the disease was discovered to have familial forms with an autosomal dominant inherited trait (Marcus et al. 1982). To date, five genes have been associated with the disease. Mutations in the plakoglobin gene are responsible for the rare autosomal recessive form of the disease also known as the Naxos disease (McKoy et al. 2000). Autosomal dominant ARVD has been linked to mutations in desmoplakin, plakophilin, desmoglein-2 and ryanodine receptor genes (Gerull et al. 2004, Pilichou et al. 2006, Rampazzo et al. 2002, Tiso et al. 2001). Mutations in plakophilin-2 (PKP2) are extremely common in the familial forms of ARVD in Europe with 70% of studied subjects carrying a mutation in this gene (van Tintelen et al. 2006).
ARVD is over-expressed in young males (Thiene et al. 1988). The first symptoms can vary from frequent premature beats or ventricular arrhythmias to signs of cardiac heart failure (Kullo et al. 1995). A definite diagnosis demands a biopsy and a histological finding of fibrofatty replacement of myocardium, but in 1994 an international consortium of ARVD scientists proposed criteria to permit the diagnosis of the disease (McKenna et al. 1994). The criteria are divided into major and minor findings and they are comprised of family history, ECG findings, arrhythmias, echocardiographic or magnetic resonance imaging findings and histological factors. The treatment strategy of ARVD begins with beta-blockers since exercise induced arrhythmias are common (Wichter et al. 1994). The only effective treatment to prohibit SCD is an ICD implantation as is the case in most inherited arrhythmias syndromes with a predisposition to SCD (Corrado et al. 2000, Fontaine & Prost-Squarcioni 2004). However, in ARVD there are major difficulties in ICD therapy (Fontaine & Prost-Squarcioni 2004). Catheter ablation has also been attempted in ARVD patients, but with mostly unsuccessful results (Fontaine et al. 2000). The reasons for unsuccessful ablation include the progressive nature and the patchy shape of the disease. Risk stratification of ARVD patients related to SCD is poor because of the lack of scientific knowledge. It is recommended that patients with aborted SCD or documented life-threatening arrhythmia or frequent syncope episodes despite of beta-blocker therapy should receive an ICD (Fontaine & Prost-Squarcioni 2004, Peters et al. 1999, Pezawas et al. 2006).

The typical ECG findings in ARVD are prolonged QRS accompanied by T-wave inversion in the right precordial leads in the primary phases of the disease and in the advanced disease right bundle branch block, left precordial lead abnormalities and epsilon waves. (Fontaine et al. 1999, Sen-Chowdhry et al. 2004) The occurrence of left ventricular involvement (decreased left ventricular ejection fraction) and prolonged QRS duration in standard ECG or signal average ECG predict VT recurrence in symptomatic ARVD patients (Peters et al. 1999, Pezawas et al. 2006).
2.2 Brugada syndrome

2.2.1 Epidemiology and characteristics

Brugada syndrome is characterized by a typical coved-type ST-segment elevation i.e. “Brugada sign” in the right precordial leads of the 12-lead ECG (Figure 1, Table 1) and it carries an elevated risk of ventricular arrhythmias (Antzelevitch et al. 2005a, Brugada & Brugada 1992, Wilde et al. 2002). A clear 8:1 male predominance in symptomatic individuals has been observed (Antzelevitch et al. 2002, Di Diego et al. 2002). The occurrence of symptoms is highest in middle-age, but the time when diagnosis is made varies from 2 days after birth to 84 years of age (Antzelevitch et al. 2005a, Antzelevitch et al. 2005b). The syndrome has been estimated to be responsible for 4-12% of all sudden deaths worldwide and at least 20% of deaths in structurally normal hearts. (Antzelevitch et al. 2002, Antzelevitch et al. 2005a)

Fig. 1. Example of type 1, 2 and 3 Brugada-type ECG patterns.
Table 1. Criteria for the Brugada syndrome type ECG.

<table>
<thead>
<tr>
<th>ECG variables</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>J wave amplitude</td>
<td>( \geq 2 \text{mm} )</td>
<td>( \geq 2 \text{mm} )</td>
<td>( \geq 2 \text{mm} )</td>
</tr>
<tr>
<td>(40ms from J-point)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T wave</td>
<td>negative</td>
<td>positive or biphasic</td>
<td>positive</td>
</tr>
<tr>
<td>ST-T configuration</td>
<td>covered</td>
<td>saddleback</td>
<td>saddleback</td>
</tr>
<tr>
<td>ST-segment</td>
<td>gradually descending</td>
<td>elevated ( \geq 1 \text{mm} )</td>
<td>elevated ( &lt;1 \text{mm} )</td>
</tr>
</tbody>
</table>

In Europe, one large ECG based study has been conducted in France with 1000 individuals where the prevalence of diagnostic type 1 Brugada ECG was found to be 0.01% (Hermida et al. 2000). This cohort was not a general population since the population was gathered from hospital ECG archives, so that the value of prevalence could be an overestimation. Type 2 or 3 Brugada ECG was found in 6% of the subjects in that study. Only three families with Brugada syndrome have been described so far in Finland with at least one additional Brugada syndrome case being diagnosed since that report appeared (Toivonen et al. 2005) (personal communication, Huikuri HV). Furthermore, there might be many undiagnosed cases of Brugada syndrome since the recognition of the entity is in its infancy in Finland.

2.2.2 Diagnosis

According to the recent consensus report (Antzelevitch et al. 2005a), only type 1 Brugada ECG pattern in more than one right precordial lead in baseline ECG or during class Ic blocker (ajmaline, flecaainide, propafenone) challenge is diagnostic for the syndrome when this is presented in a patient with one of the following features: documented VF, polymorphic VT, a family history of sudden cardiac death at <45 years old, type 1 Brugada ECGs in family members, inducibility of VT with programmed electrical stimulation, syncope, or nocturnal agonal respiration. The concealed form of Brugada ECG can be unmasked with drug challenge but also during fever. Drug challenge should not be performed in patients with baseline type I Brugada ECG in any documented ECG tracing since very limited additional diagnostic or prognostic information can be gained from the test and the test can provoke lethal arrhythmias. The drug challenge should only be used in patients with symptoms related to the disease (see above) where Brugada ECG pattern is suspected (type 2 or 3 Brugada ECG) since there are still risks associated with the challenge.
The differential diagnosis of Brugada syndrome is rather difficult since many conditions can mimic the Brugada ECG pattern; a list is provided in table 2. The initial differential diagnostic examinations after a resuscitated arrhythmia are echocardiography and angiography in order to exclude all structural abnormalities that might predispose to ventricular arrhythmias. These examinations should reveal most of the clinical conditions mentioned in the differential diagnosis table.

From the differential diagnosis point of view, ARVD is the most difficult disease to distinguish from Brugada syndrome. This is a significant problem since the first line treatment is so fundamentally different. In ARVD, beta-blockers are effective to some extent, but in Brugada syndrome beta-blockers are contraindicated. (Antzelevitch et al. 2005a) Both ARVD and Brugada syndrome can cause repolarization abnormalities in right precordial leads, both predispose to ventricular tachyarrhythmias and in ARVD the fatty deposits can be so minimal that they may not be detectable. One possibility for providing a differential diagnosis in this situation is the arrhythmia trigger. In Brugada syndrome, arrhythmias occur usually during vagotonic states whereas in ARVD usually during exercise (Antzelevitch et al. 2005b, Pezawas et al. 2006). In addition, in LQT3, arrhythmias may often occur during vagotonic states, but in this case the ECG pattern differs greatly from that seen in the Brugada syndrome ECG (Ackerman 1998).

Table 2. Differential diagnosis of Brugada syndrome ECG pattern.

<table>
<thead>
<tr>
<th>Differential diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right bundle branch block</td>
</tr>
<tr>
<td>Left ventricular hypertrophy</td>
</tr>
<tr>
<td>Acute pericarditis</td>
</tr>
<tr>
<td>Acute myocardial ischemia or infarction</td>
</tr>
<tr>
<td>Early repolarization</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
</tr>
<tr>
<td>Aortic dissection</td>
</tr>
<tr>
<td>Hyperkalemia</td>
</tr>
<tr>
<td>Hypercalcemia</td>
</tr>
<tr>
<td>Arrhythmogenic right ventricular dysplasia</td>
</tr>
<tr>
<td>Hypothermia</td>
</tr>
<tr>
<td>Mechanical compression of the right ventricular outflow tract</td>
</tr>
<tr>
<td>Hemopericardium</td>
</tr>
</tbody>
</table>
2.2.3 Genetic background

Brugada syndrome is an autosomal dominant disease with variable penetrance. At present, four genes have been associated with the disease. Mutations in SCN5A gene are found in approximately 20% of all Brugada syndrome patients (Antzelevitch et al. 2005a, Chen et al. 1998). All mutations in Brugada syndrome cause a loss-of-function in the cardiac sodium channel by directly affecting the protein itself or by interfering with the trafficking of the protein to the cell membrane (Chen et al. 1998). Recently, mutations in the gene GPD1-L have been associated with Brugada syndrome. Mutations in this particular gene interfere also with sodium channel function and result in the same cascade of electrophysiological characteristics. (London et al. 2007) Additionally, loss-of-function in calcium channel encoding genes CACNA1C and CACNB2 have been linked to overlapping Brugada and SQT syndrome (Antzelevitch et al. 2007).

2.2.4 Electrophysiology and arrhythmias

The electrophysiological background for the characteristic “Brugada sign” can be traced to a sodium channel defect. The amount of the sodium inflow to the cell is decreased but all other ion channels work appropriately. In phase 2 of the action potential, it is the $I_{to}$ channel which starts the repolarization process. In areas where $I_{to}$ is predominant, such as the right ventricular epicardium, one can see an increased notch in the action potential curve should the sodium channel function be impaired and a current appears between the epicardium and the endocardium of the right ventricle which results in a J-wave ST-segment elevation in the surface ECG (Figure 2). (Antzelevitch 2001, Kimura et al. 2004) Administration of class Ic sodium channel blocker will accentuate the sodium channel abnormality and thus these drugs are used to aid the diagnosis of Brugada syndrome in unclear situations such as type 2 or 3 Brugada ECG (Brugada et al. 2000). Fever may also unmask the Brugada ECG, since higher temperatures decrease the sodium channel flow (Porres et al. 2002).
Ventricular arrhythmias in Brugada syndrome are generated by a phase 2 re-entry mechanism. Due to the notch created by Ito current, sometimes the plateau dome from the epicardium disappears and a strong current between endocardium, other epicardial areas and the particular repolarized epicardial area can be generated. This current produces a new action potential in the repolarized area even though other areas of the cardiac muscle are still in repolarization phase and later on this action potential difference causes a current to the normally repolarized area and a re-entry circuit will be created (Figure 3). (Antzelevitch 2001, Kimura et al. 2004) Usually the presenting arrhythmia is either polymorphic VT or VF but about 20% of the patients exhibit also atrial arrhythmias (Bordachar et al. 2004, Eckardt et al. 2001, Itoh et al. 2001) and a prolonged sinus node recovery and atrioventricular conduction time have also been described (Morita et al. 2004, Takehara et al. 2004). There are three unique clinical characteristics to Brugada syndrome VF occurrence. First, in Brugada syndrome patients, VF is usually
triggered by very short coupled premature ventricular complexes (PVC) even though patients with the syndrome rarely display PVCs in their ambulatory recordings. Second, VF episodes occur mostly during sleep (Itoh et al. 2001). Third, VF episodes are commonly self-terminating, which is uncommon in other disease entities, but also seen typically in LQT3. Therefore, Brugada patients may present with symptoms such as syncope or sleep disturbances. The high occurrence of atrial arrhythmias, mainly AF, can cause problems in prognostic studies such as EP testing and in treatment with ICD due to the inappropriate shock possibility. Brugada syndrome patients with atrial arrhythmias seem to have a poorer outcome (Bordachar et al. 2004).

![Monophasic action potentials and surface ECG illustration of phase 2 re-entry.](image)

*Fig. 3. Monophasic action potentials and surface ECG illustration of phase 2 re-entry. Endo= endocardium, Epi 1= epicardial area 1, Epi 2= epicardial area 2, ECG = surface ECG.*
2.2.5 Inductors of Brugada ECG

Several nongenetic factors have been mentioned in the literature as possible inductors of the ECG pattern resembling Brugada syndrome. As such, a Brugada-type ECG may appear in some patients during febrile states and in patients who are under the influence of cocaine or pharmaceutical drugs that have a sodium channel blocking effect, e.g. certain antiarrythmic drugs, some anesthesics and tricyclic antidepressants. (Aksay et al. 2005, Bebarta & Summers 2007, Brugada et al. 2006, Goldgran-Toledano et al. 2002, Porres et al. 2002, Vernooy et al. 2006) Propofol has also been claimed to evoke the appearance of a pattern of Brugada syndrome (Vernooy et al. 2006a), however, its pathophysiologic mechanism remains unclear. The clinical meaning and the risk of arrhythmias induced by this pattern are unknown.

2.3 Risk stratification among patients with Brugada syndrome

2.3.1 ECG and clinical examinations

Patients with prior symptoms such as aborted SCD, syncope or documented ventricular arrhythmia are at a high risk of symptom recurrence and SCD. Likewise, patients with a Brugada type 1 ECG pattern in their baseline ECG have been associated with higher risk of SCD than subjects with type 1 Brugada ECG after class Ic antiaarrythmic drug challenge. Males have been reported to have five fold risk of ventricular arrhythmias in the syndrome but a family history of the disease does not predict the outcome. (Brugada et al. 2002, Brugada et al. 2003) Many non-invasive risk stratifying markers such as late potentials in signal average ECG, fluctuation of the typical “Brugada sign” in the ECG, transmural dispersion of repolarization measurement from the ECG, higher J-point amplitude in surface ECG have been associated with a higher risk of symptoms in Brugada syndrome. (Castro Hevia et al. 2006, Eckardt et al. 2005, Ikeda et al. 2001, Ikeda et al. 2005, Veltmann et al. 2006)

2.3.2 Electrophysiological testing

One rather intriguing question lies in the use of prophylactic ICD in Brugada syndrome. The inducibility of sustained ventricular tachyarrhythmias during EP testing has been used as a tool in risk stratification (Brugada et al. 2003,
Nademanee 2002). However, the value of the test is controversial because some recent studies have reported negative results (Eckardt et al. 2005, Gehi et al. 2006, Priori et al. 2002a). One large study has been conducted where 160 asymptomatic Brugada syndrome patients underwent an electrophysiological evaluation. During a prospective follow-up period of over 24 months, those study subjects that were not inducible to VT/VF did not experience life-threatening events, whereas those who were inducible experienced a 2% incidence of these events (personal communication, Brugada P).

2.3.3 Genetic modulators

All mutations in the SCN5A gene that cause the syndrome create a loss-of-function in the sodium channel encoded by the gene. The loss-of-function is generated when the mutation interferes with the protein directly, or it may interfere with the trafficking of the protein to the cell membrane thus preventing the emergence of the sodium current or causing the channel to activate or inactivate abnormally (Antzelevitch 2001, Balser 2001, Priori et al. 2002a, Tan et al. 2003). A mutation of the SCN5A has been found in 18-30% of the cases and a second locus besides SCN5A has been identified in chromosome 3 (Weiss et al. 2002). From this new locus the responsible gene, GPD1L, has been detected recently as well as the calcium channel genes (London et al. 2007, Antzelevitch et al. 2007). The genotype-phenotype characteristics or the prevalence of mutations in these genes causing Brugada syndrome are unknown. A variety of SCN5A related abnormalities could be also in the background of the disease since promoter area differences, cryptic splicing mutations or gross rearrangements in the gene have been scantily studied. The present knowledge on mutations has not helped in the prognostic evaluation since many patients without SCN5A mutation present the same rate of adverse events as the SCN5A mutation carriers (Gehi et al. 2006). Screening of the SCN5A is still warranted as it helps in the early detection of the syndrome in a family member of a SCN5A mutation carrier and it does provide more information on the genotype-phenotype relationship for the future Brugada syndrome studies.

The human genome has over 10 million common variants that are called single nucleotide polymorphisms (SNP) and these variants contain most of the variability of the human genome. Many of these SNPs are thought to modulate the gene-protein and genotype-phenotype relationship in human physiology. SNP is basically a mutation but the frequency of the SNP is over 1% in the population.
In previous studies three common genetic modulators for Brugada syndrome have been described, namely H558R, R1193Q and a common promoter haplotype in SCN5A gene (Bezzina et al. 2006, Huang et al. 2006, Niu et al. 2006, Poelzing et al. 2006). The R1193Q variant increases the loss-of-function effect of the SCN5A mutation in the sodium channels, resulting in even more reduced current. The R1193Q variant has also been associated with the long QT syndrome (Huang et al. 2006). Likewise, the decreased expression caused by a common haplotype in the promoter area of SCN5A gene supports the concept of modulation of the disease in Japanese subjects (Bezzina et al. 2006). The role of common variations present in the SCN5A gene is still poorly described. A single variation, S1103Y, has been associated with an increased risk of arrhythmias in the African American population and with an increased risk of Sudden Infant Death Syndrome (Plant et al. 2006). This publication can be considered as proof of concept of the role of common variations in the predisposition to sudden death phenotypes. Another variation, H558R, is present in 20% of the white population (Ackerman et al. 2004). In vitro studies have shown that H558R modulates the effects of nearby mutations even when the variation and the mutation are located on a different allele (Poelzing et al. 2006, Viswanathan et al. 2003, Ye et al. 2003). Only the SCN5A common promoter haplotype has been studied in a larger Brugada syndrome population with respect to its association to clinical outcomes and ECG findings (Bezzina et al. 2006).

### 2.4 Familial atrial fibrillation

#### 2.4.1 Epidemiology

AF is the most common sustained arrhythmia (Tsang et al. 2005) and it accounts for 15% of all strokes (Wolf et al. 1991). The prevalence of AF is highly dependent on age since the incidence increases with age, from 1% in young adults to approximately 10% of those over 80 years old (Kannel et al. 1998). AF is frequently the result of diverse cardiac or systemic disorders including hypertension, coronary disease, valvular diseases, cardiomyopathies and thyreotoxicosis (Fuster et al. 2001). However, in 10-20% of AF patients, no underlying disease or precipitating factors can be found (Brand et al. 1985, Patton et al. 2005). In this situation the terms “lone AF” or “idiopathic AF” are used
illustrating the endogenous patophysiology of the arrhythmia. In this patient group, it is hoped that genetics will become soon an important etiological factor.

The first familial case of AF was described in 1936 and recently several studies have evaluated the prevalence of familial aggregation of AF (Arnar \textit{et al.} 2006, Ellinor \textit{et al.} 2005, Fox \textit{et al.} 2004). The prevalence of familial AF, i.e. at least one first- or second-degree family member affected, in lone AF group of 110 patients was 38\% (Ellinor \textit{et al.} 2005) and in larger epidemiological studies e.g. the Framingham Heart Study the odds ratio of having AF if a brother had lone AF was as high as 70 times (Fox \textit{et al.} 2004). In an Icelandic study, the possibility of having AF was elevated five fold if one first-degree relative had suffered AF before the age of 60 (Arnar \textit{et al.} 2006).

2.4.2 Genetics

The first genetic evidence of inherited AF was described in 1997 by Brugada \textit{et al} with a locus in 10q22-24 that was present in five Spanish AF families (Brugada \textit{et al.} 1997). Subsequently, several loci for AF have been identified in chromosomes 6, 11, 12, 17 and 21. Subsequently, several gene mutations have been associated with familial AF. The first gene connected with the disease was KCNQ1, but in the affected family, a long QT interval was also present (Chen \textit{et al.} 2003). Another mutation in KCNQ1 gene was also discovered and this did not interfere with QT interval (Otway \textit{et al.} 2007). Mutations in three other genes, namely KCNA5, KCNJ2, KCNE2, have also been described (Olson \textit{et al.} 2006, Xia \textit{et al.} 2005, Yang \textit{et al.} 2004). Also three other genes (LMNA, SCN5A, KCNH2) have been associated with familial AF but as a secondary phenotype after conduction disease (SCN5A, LMNA) or short QT syndrome (KCNH2) (Fatkin \textit{et al.} 1999, Hong \textit{et al.} 2005, McNair \textit{et al.} 2004, Sebillon \textit{et al.} 2003). A common factor in the clinical features seem to be early onset AF (~< 40years) without underlying causes.

2.5 Summary

In summary, Brugada syndrome, LQT and SQT syndromes are considered as primary channelopathies and one could claim CPVT as secondary channelopathy without cardiac structural abnormalities. Familial lone AF might turn out to be a channelopathy at least some subtypes of the disorder. HCM and ARVD are arrhythmia syndromes with structural abnormalities. In Brugada syndrome, the
prevalence of the disease is very unevenly distributed in respect of geography and the prevalence of the syndrome or the ECG finding in Finland or Scandinavia is not known. Additionally, the risk stratification of patients with Brugada type ECG has not been uniformly established.
3  Purpose of the present study

The purpose of the present study was to examine the Brugada type ECG pattern in different patient populations and to evaluate the risk of life-threatening arrhythmias among Brugada syndrome patients by utilizing genetic techniques and recording their ECG. The specific aims were:

1. To assess the prevalence of different Brugada type ECG patterns and to evaluate the prognostic significance of these findings in two Finnish populations (I)
2. To examine the relationship between Brugada type ECG and inherited AF according to our anecdotal findings (II)
3. To evaluate the relationship of standard ECG measurements and symptoms among patients with Brugada syndrome (III)
4. To evaluate the significance of the variant H558R among Brugada syndrome patients with respect to symptoms and ECG findings (IV)
5. To evaluate the risk of life-threatening arrhythmias among patients with induced Brugada ECG pattern during an acute medical event (V)
4 Study populations

4.1 Study population I

In the study of prevalence and prognosis of Brugada type ECG in Finland we examined two populations. The first study population group consisted of 2479 young male subjects who had applied for enlistment in the Finnish Air Force between 1980–1990 (age range 18–30 years), and the second group consisted of a randomly selected population of 542 healthy subjects (age 40–60 years, mean 50±6 years, 274 males and 268 females) (I).

4.2 Study population II

In the second study (II) the lone AF population was selected among 220 consecutive patients admitted into Oulu University Hospital for treatment of acute AF or catheter ablation of AF during the years 2002 and 2003. Of them, a total of 168 patients consented to participate in the study. Their mean age was 50 ± 8 years (range 20–63 years), and 130 (77%) of them were male. All subjects with a history of any cardiovascular, pulmonary (e.g. asthma), or metabolic (e.g. hyperthyroidism and diabetes) disease or with hypertension were excluded. Other exclusion criteria were electrical or medical cardioversion within 30 days before the examination, history of thoracic surgery or radio frequency catheter ablation, permanent pacemaker, and any of the following echocardiographic findings: depressed left ventricular systolic function (ejection fraction <50%), abnormal wall motion of the left ventricle, septal hypertrophy (>15 mm), left atrial size >50 mm, and significant valve abnormality. The middle-aged population from study I was used as a control population. Although one subject had to be excluded from the control population since he had type 2 Brugada ECG and early onset AF, we only used the control population for ECG prevalence comparison. The study arrangement for familial AF comparison was saddleback type ST-segment elevation (type 2 or 3 Brugada ECG) subjects (cases, n= 17) with AF versus AF subjects without the ECG abnormality (controls, n=151).
4.3 Study population III-V

In the third and fourth study (III-IV) we analyzed ECGs from an international Brugada syndrome database which includes data from 464 patients. In the third study, (III) we analyzed 12-lead ECGs’ from 200 consecutive probands with spontaneous type I Brugada ECG at baseline. In addition, we performed a cross-sectional case (symptomatic, n= 66)-control (asymptomatic, n= 134) study in Brugada syndrome subjects (one baseline 12-lead ECG per subject). Their mean age at diagnosis was 42 ± 16 years and 142 (62%) subjects were males (Table 4, results section). All ECGs’ were collected from the date of diagnosis. From the same database of 464 subjects, we conducted a subanalysis of subjects that had had the H558R variant sequenced (IV) and a high quality 12-lead ECG had been obtained. We were able to evaluate data from 75 subjects with the SCN5A mutation and 92 subjects without SCN5A mutations. Their mean age at diagnosis was 39 ± 15 and 42 ± 17 years, and 65% and 86% were male, respectively. From the same database we gathered all cases (n=47) of induced Brugada type 1 ECG during an acute medical event and examined their clinical symptoms during the event (V).
5 Study protocols and methods

5.1 Brugada type ECG in Finland (I)

All Air Force applicants underwent a thorough physical examination and 12-lead ECG (at paper speed 50 mm/s and 1 mV/10 mm standard gain) in 1980–1990. The subjects of the middle-aged population underwent thorough physical examination, two-dimensional and M-mode echocardiography, and extensive laboratory tests in 1991–1992 at Oulu University Hospital. The ECG criteria proposed at that time by the Study Group on the Molecular Basis of Arrhythmias of the European Society of Cardiology was used to identify the subjects with the Brugada type ECG (Wilde et al. 2002) (Figure 1). All ECGs were retrospectively reviewed by two independent investigators to assess the prevalence of the Brugada type ECG. A summary of the criteria used for the subclassification of ECG abnormalities into types 1, 2, or 3 is shown in Table 1.

Subjects with the Brugada type ECG in the younger population were contacted in June 2003 and those of the middle-aged population in December 2003 by telephone or by a mailed questionnaire after a mean retrospective follow-up period of 19±2 years (range 13–23 years) in the Air Force applicant study population and after a mean follow-up period of 11±1 year in the population of healthy middle-aged subjects (range 11–12 years). The subjects were asked about symptoms and any possible family history of arrhythmias (e.g., palpitations, syncope). In those cases where there were arrhythmia symptoms, the type of arrhythmia was validated from the ECG recordings and/or hospital records.

5.2 Brugada type ECG in lone atrial fibrillation (II)

The prevalence of Brugada type was evaluated because of our clinical anecdotal data on some families with Brugada type ECG pattern and the familial form of lone AF. We reviewed all the 12-lead ECGs (paper speed 50 mm/s and 1 mV/10 mm standard gain). The same diagnostic criteria as in study I were used to identify the subjects with Brugada type ECG (Antzelevitch et al. 2005a). The authors of the Brugada syndrome consensus report recommended the use of diagnostic drug challenge with a class IC antiarrhythmic agent to unmask the coved-type ST-segment elevation among the subjects with type 2 or 3 ECG changes in the baseline ECG (Antzelevitch et al. 2005a). Therefore, a diagnostic
drug challenge with flecainide (150 mg or 2 mg/kg over 10 min) or ajmaline (50 mg or 1 mg/kg over 5 min) was offered to all the subjects with a Brugada type ECG and a history of AF episodes. Thirteen subjects (76%) consented to undergo the test.

The family history of AF, syncope, and VT was evaluated by the individuals’ responses to a mailed questionnaire. The information was verified by phone conversation and original patient records were acquired from the referring hospitals and primary care physicians. The time of the first AF episode, total number of symptomatic AF episodes, and a complete family history of documented AF episodes were obtained from all patients. The family history of AF was considered positive if at least one of the first-degree family members (i.e. parents, siblings, or children) had had ECG-documented AF episode(s). A clear family history of AF, suggesting autosomal dominant inheritance, was defined as documented AF in >30% of the first-degree family members.

One family with 19 siblings had an extraordinary high occurrence of lone AF and Brugada type ECG (Figure 4). In this family, the SCN5A gene that has been previously described in the Brugada syndrome was screened from the affected proband of the family. Genomic DNA was isolated from peripheral blood leukocytes using the commercial kit (Gentra System, Puregene, Minneapolis, MN, USA). Exons of the SCN5A were amplified and analysed by direct sequencing, using primers designed from the published gene sequence. Polymerase chain reaction products were purified with a commercial reagent (ExoSAP-IT, USB, Cleveland, OH, USA) and directly sequenced from both directions with an ABI PRISM 3100-Avant Automatic Sequencer (Foster City, CA, USA).
Fig. 4. Pedigree of a family with a high prevalence of lone AF and saddleback-type ST-segment elevation. Females are represented by circles and males by squares. Subjects with AF are designated by filled symbols. In addition to the proband (marked with an arrow), five other siblings and the father of the siblings (who also had lone AF) had saddleback-type ST-segment elevation and these subjects are marked with a star under the symbol.

5.3 ECG features and genetics among patients with Brugada syndrome (III-V)

The assessment of the ECGs in the study population was conducted blinded to symptoms. From standard 12-lead ECG leads II and V2 PR-, QRS-, QT-, Tpeak-Tend (TpTe) –intervals were measured. Also R'/S – wave amplitude ratio from lead aVR (“aVR sign”), QRS electrical axis from the limb leads and highest J-point elevation amplitude from leads V1-3 were evaluated. The axis of the QRS and “aVR sign” were analyzed as additional information since, according to the anecdotal data from our research groups; a higher “aVR sign” and the occurrence of left axis deviation were thought to be different between symptomatic and asymptomatic groups. Furthermore, PR- and QRS-intervals were measured with standard methods. The J-point as the end-point of the QRS-interval was determined as a consensus from all the precordial leads and also from lead II due to the obvious difficulties of J-point determination from lead V2. QT-interval was measured by the standard tangential method and corrected for heart rate with
Bazetts’ equation (QTc). TpTe-interval was measured from the highest tip of the T-wave to the end of the T-wave.

Subjects with prior syncope, aborted SCD or documented ventricular tachyarrhythmia were considered as symptomatic subjects. In study III, an evaluation of the ECG was made between symptomatic and asymptomatic subjects and in study IV the evaluation of ECG and symptoms was made between H558R H allele and R allele carriers. The genotyping of variant H558R was performed with direct sequencing with the same protocol as in study II.

We collected data on 47 patients (69% male, mean age 48 ± 16 years) who presented during an acute medical event with a typical Brugada-type ECG, i.e. that meeting the criteria of the ESC consensus report task force (Antzelevitch et al. 2005a). We used the clinical histories, follow-up visit reports and blood samples for research and genetic diagnostic purposes from those subjects who agreed to participate in the genetic screening. The cases were obtained either through direct contact from the patients themselves or from their attending physicians for further clinical management and genetic advice.

5.4 Statistical analysis

The data were analysed using SPSS 10.1 software (I-III) (SPSS Inc, Chicago, IL) and SAS v9.1.3 (IV) (SAS, Cary, NC). The statistical data is presented as mean ± standard deviation, as 95% confidence interval (CI) or as median with range in unequal distribution groups. Interobserver agreement was determined by the overall proportion of agreement and the Kappa statistics. The statistical significance of the differences between the population groups was analysed with Chi-square test or Fisher exact test for categorical variables and t-test for continuous variables with a normal distribution. When the distribution was skewed, non-parametric tests (two-sided Mann-Whitney test or Wilcoxon signed rank test) were used.

Logistic regression analysis for binary logistics was used for odds ratio (OR) determination. Receiver operating characteristics (ROC) curves were generated for variables that differed between the groups in the analysis. Curve point with the highest sum of specificity and sensitivity was labelled as the optimized cut-off point and used in the calculation of OR, sensitivity and specificity analyses. All tests were two sided and statistical significance was set at p< 0.05.
6 Results

6.1 Prevalence and prognosis of Brugada type ECG pattern in Finnish populations (I)

We examined the ECGs of 2479 healthy Air Force applicants and identified 15 subjects (0.61%, 95%CI: 0.30–0.91%) with a Brugada-type ECG pattern. Both investigators found 17 ECGs that fulfilled the criteria for type 2 or 3 Brugada syndrome but neither of the investigators detected any subjects with type 1 Brugada ECG abnormality. The overall proportion of agreement was 88% with a Kappa score of 0.59 and only two subjects were excluded because of the lack of interobserver agreement. Hence, all the subjects with the Brugada type ECG in our population displayed a saddleback-type ECG abnormality. The mean age of the subjects with the Brugada type ECG was 20±3 years. In the second study population of 542 subjects, both investigators found three subjects (0.55%, 95%CI: 0.07–1.18%) (two males and one female) with a Brugada-type ECG pattern. These three cases were independently identified by both investigators (100% agreement).

The prognosis of the subjects with the Brugada type ECG in the Air Force study population was excellent as they all were still alive 19±2 years after the initial examination. Moreover, none of the subjects had experienced syncope or life-threatening ventricular tachyarrhythmias, and there were no reports of a family history of SCD or life-threatening arrhythmias. Likewise, during the retrospective follow-up (11±1 years), none of the three subjects with the Brugada type ECG in the middle-aged population had died, or had suffered syncope or life-threatening ventricular tachyarrhythmias. There were no family histories of sudden cardiac death. One subject had started to exhibit frequent paroxysmal AF episodes, documented by ECG, five years after the initial examination.

6.2 Familial clustering of lone atrial fibrillation patients with saddleback type ST-segment elevation (type 2 or 3 Brugada ECG) (II)

The prevalence of Brugada type ECG was significantly higher among the patients with lone AF (17/168, 10%, 95% CI: 6–15%) than the healthy control subjects (2/541, 0.4%, 95% CI: 0–0.9%) (p<0.001) (Figure 5). None of the patients with
lone AF had coved-type ST-segment elevation (malignant type 1 Brugada ECG) at baseline ECG or after drug challenge with flecainide (n = 8) or ajmaline (n = 5) (Figure 6).

![Graph](image)

**Fig. 5. Prevalence of saddleback-type ST-segment elevation in patients with lone AF and healthy control subjects.** The prevalence of saddleback-type ST-segment elevation among patients with lone AF is depicted with the column on the left (10%, 95% CI: 6–15%), whereas the column on the right represents the control population (0.4%, 95% CI: 0–0.9%). p < 0.001 lone AF vs. control.
Fig. 6. Typical example of saddleback-type ST-segment elevation in patient with lone AF in baseline ECG and during drug challenge. The precordial leads (V1–V6) of a subject who presented with a saddleback-type elevation of the J-point and the ST-segment at baseline ECG (A) and during drug challenge with flecainide (B) are also shown. Gain 10 mm/mV and paper speed 50 mm/s.

The clinical characteristics and echocardiographic data are presented in Table 3. With respect to the patients with lone AF, there were no differences in the duration of AF history (p = 0.17), age at AF onset (p = 0.10), total number of symptomatic AF episodes (p = 0.73), fractional shortening (p = 0.79), or LA size (p = 0.77) between the subjects with Brugada type ECG and those with normal ECG.

Many patients with lone AF and Brugada type ECG had familial clustering of lone AF. Overall, 59% of the patients with Brugada type ECG and lone AF had at least one of their first-degree family members (i.e. parents, siblings, or children) with ECG-documented AF episode(s). In those patients with lone AF but no ECG abnormalities, the family history for lone AF was positive in 36% of the cases (p = 0.07). A clear family history of AF (>30% of first-degree relatives with AF episodes) was significantly more common among the patients with Brugada type ECG (24 vs. 7%, p = 0.03). In one large family with 19 siblings, 11 had AF and
six of them exhibited type 2 Brugada ECG (Figure 4). This kind of pedigree suggests an autosomal dominant pattern of inheritance (Figure 4). Genetic analysis of the proband in this particular family did not identify any mutations in the SCN5A gene. Therefore, all mutations in this gene that may cause Brugada syndrome have been excluded in this family. None of the patients with lone AF and Brugada type ECG had a personal or family history of SCD, life-threatening ventricular arrhythmias, or syncope.

Table 3. Clinical characteristics of the patients with lone AF.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal ECG</th>
<th>Brugada type ECG</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>50±8</td>
<td>49±9</td>
<td>NS</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>116 (77 %)</td>
<td>14 (82 %)</td>
<td>NS</td>
</tr>
<tr>
<td>female</td>
<td>35 (23 %)</td>
<td>3 (18 %)</td>
<td></td>
</tr>
<tr>
<td>AF history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age at AF onset, yrs</td>
<td>41±10</td>
<td>37±10</td>
<td>NS</td>
</tr>
<tr>
<td>duration of AF history, yrs</td>
<td>9±7</td>
<td>11±9</td>
<td>NS</td>
</tr>
<tr>
<td>number of symptomatic AF episodes</td>
<td>44±105</td>
<td>23±105</td>
<td>NS</td>
</tr>
<tr>
<td>AF in at least one family member</td>
<td>55 (36%)</td>
<td>10 (59%)</td>
<td>p=0.07</td>
</tr>
<tr>
<td>AF in &gt;30% of family members</td>
<td>11 (7%)</td>
<td>4 (24%)</td>
<td>p=0.03</td>
</tr>
<tr>
<td>Echocardiographic findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA diameter, mm</td>
<td>40±5</td>
<td>41±5</td>
<td>NS</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>36±11</td>
<td>37±9</td>
<td>NS</td>
</tr>
</tbody>
</table>

LA= left atrial, NS= not significant

6.3 Differences in 12-lead ECG between symptomatic and asymptomatic Brugada syndrome patients (III)

Only male gender was found to be significantly more frequent among symptomatic subjects in the demographical data (Table 4). Age or occurrence of an SCN5A mutation did not correlate with symptoms.

With respect to the ECG measurements, QRS duration in lead II and V2 differed significantly between symptomatic versus asymptomatic subjects (in lead II: mean 109 ± 26 ms vs. 100 ± 21 ms, p=0.006; in lead V2: mean 115 ± 26 ms vs. 104 ± 19ms, p< 0.001). Interestingly, all subjects had the highest J-point elevation amplitude in lead V2 and the measurements were made from this lead and J-point elevation was not found to be significantly different between groups. The mean values of ECG measurements are presented in detail in table 5.
An optimized cut-off point was determined from the receiver operating characteristic (ROC) curve for QRS duration in lead V2 in respect to symptoms. The optimized cut-off point for QRS duration was 120ms in this lead to a specificity of 70% and sensitivity of 52% to differentiate asymptomatic from symptomatic subjects. The OR of being symptomatic was 2.5 (95% CI: 1.4 - 4.6, p=0.003) if the QRS duration was ≥ 120ms in lead V2. In the regression analysis of QRS ≥120ms in lead V2 adjusted for gender, age and the presence of SCN5A mutation, the OR was 2.6 (95% CI: 1.4 – 4.8, p=0.004). When excluding syncope from the symptomatic patients, the OR for prior sustained VT, VF or SCD with QRS ≥120ms in lead V2 was 2.7 (95% CI: 1.3 – 5.8, p=0.011). The SCN5A mutation carriers displayed a trend towards longer QRS in lead V2 compared to the SCN5A negative subjects (113ms ± 25ms vs. 103 ± 19ms, p=0.125) (Table 5).

Table 4. Differences in characteristics between symptomatic and asymptomatic Brugada syndrome subjects.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Symptomatic (n=66)</th>
<th>Asymptomatic (n=134)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>88% male</td>
<td>63% male</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>40 ± 15 years</td>
<td>40 ± 16 years</td>
<td>0.392</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Syncope n=33;</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VT/VF n=6;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCD n=27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCN5A mutation</td>
<td>53%</td>
<td>47%</td>
<td>0.378</td>
</tr>
<tr>
<td>(analyzed n=51)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VT/VF= Ventricular tachycardia or ventricular fibrillation; SCD= Sudden cardiac death

Table 5. ECG Measurements between symptomatic and asymptomatic Brugada syndrome subjects.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Symptomatic</th>
<th>Asymptomatic</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR II</td>
<td>181 ± 34ms</td>
<td>178 ± 39ms</td>
<td>0.605</td>
</tr>
<tr>
<td>PR V2</td>
<td>170 ± 38ms</td>
<td>171 ± 38ms</td>
<td>0.856</td>
</tr>
<tr>
<td>QRS II</td>
<td>109 ± 26ms</td>
<td>100 ± 21ms</td>
<td>0.006</td>
</tr>
<tr>
<td>QRS V2</td>
<td>115 ± 26ms</td>
<td>104 ± 19ms</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>QTc II</td>
<td>414 ± 68ms</td>
<td>404 ± 71ms</td>
<td>0.395</td>
</tr>
<tr>
<td>QTc V2</td>
<td>430 ± 70ms</td>
<td>417 ± 79ms</td>
<td>0.267</td>
</tr>
<tr>
<td>TpTe II</td>
<td>79 ± 20ms</td>
<td>77 ± 18ms</td>
<td>0.433</td>
</tr>
<tr>
<td>TpTe V2</td>
<td>88 ± 29ms</td>
<td>83 ± 23ms</td>
<td>0.197</td>
</tr>
<tr>
<td>J-point V2</td>
<td>0.36 ± 0.18mV</td>
<td>0.40 ± 0.22mV</td>
<td>0.236</td>
</tr>
<tr>
<td>“aVR sign”</td>
<td>0.53 ± 0.71</td>
<td>0.41 ± 0.48</td>
<td>0.189</td>
</tr>
<tr>
<td>Left axis deviation</td>
<td>29%</td>
<td>23%</td>
<td>0.427</td>
</tr>
</tbody>
</table>

“aVR sign” = R’/S wave amplitude ratio from lead aVR; TpTe = T-wave peak to T-wave end interval.
6.4 Common variant H558R as a genetic modulator in Brugada syndrome (IV)

The alleles of H558R (A→G) locus were in Hardy-Weinberg equilibrium, in the whole population as well as in both study groups. Twenty-four subjects (30%) had symptoms related to Brugada syndrome in the SCN5A mutation group and 44 subjects (46%) had symptoms in the non-SCN5A mutation group. The G allele frequency was 31% of SCN5A mutation carriers and 16% in non-SCN5A mutation patients.

The AA genotype carriers had a trend towards more symptomatic subjects compared to AG and GG carriers (50% v. 27%, p= 0.067) in the SCN5A mutation group. From the ECG measurements, QRS duration in lead II was significantly longer in AA genotype carriers compared to AG or GG carriers (p= 0.017) in this group. Also “aVR sign” and naturally R’ wave amplitude were significantly higher (p= 0.005; p= 0.004, respectively) in subjects with the AA genotype as was J-point elevation amplitude (p= 0.013). The detailed ECG measurements of SCN5A mutation group are presented in table 6. None of the ECG or any of the clinical parameters were significant between the genotyped groups in individuals without a mutation in SCN5A.

Table 6. The SCN5A mutation group ECG measurements between genotype groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median and range AA</th>
<th>Median and range AG and GG</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>J point V2</td>
<td>0.5mV (0.2-1mV)</td>
<td>0.3mV (0.2-1mV)</td>
<td>0.013</td>
</tr>
<tr>
<td>PR II</td>
<td>200ms (130-320ms)</td>
<td>190ms (140-280ms)</td>
<td>0.522</td>
</tr>
<tr>
<td>PR V2</td>
<td>200ms (120-320ms)</td>
<td>180ms (140-280ms)</td>
<td>0.300</td>
</tr>
<tr>
<td>QRS II</td>
<td>120ms (80-200ms)</td>
<td>100ms (60-180ms)</td>
<td>0.017</td>
</tr>
<tr>
<td>QRS V2</td>
<td>120ms (80-180ms)</td>
<td>100ms (80-180ms)</td>
<td>0.162</td>
</tr>
<tr>
<td>QTc II</td>
<td>413ms (348-496ms)</td>
<td>423ms (298-539ms)</td>
<td>0.252</td>
</tr>
<tr>
<td>QTc V2</td>
<td>443ms (340-518ms)</td>
<td>440ms (323-581ms)</td>
<td>1</td>
</tr>
<tr>
<td>“aVR sign”</td>
<td>0.655 (0-3)</td>
<td>0.33 (0-2.5)</td>
<td>0.005</td>
</tr>
<tr>
<td>R’ aVR</td>
<td>0.3mV (0-4.5mV)</td>
<td>0.15mV (0-5mV)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

The “aVR sign” is the ratio of R’/S wave amplitudes from lead aVR. Results are presented as median with range because distributions of all measures were skewed. Significant p values are in bold letters. Wilcoxon signed rank test was used in the analysis with normal approximation and continuity correction of 0.5. Range presented in parenthesis.
6.5 Induced Brugada ECG (V)

In 16 patients a Brugada-type ECG was observed during a febrile episode. In 26 patients, the ECG abnormality was induced because of a drug/medication (e.g. cocaine, anesthetics, antiarrhythmics, antidepressants, antihistamines), and 5 were due to electrolyte imbalances. Of the 47 patients with an acute Brugada-type ECG, 24 (51%) had suffered malignant arrhythmias, and of these 18 patients developed SCD (or aborted SCD), three subjects had ventricular tachycardia (VT) episodes, and three experienced syncope. Of the 18 patients who developed sudden cardiac death, six were associated with febrile episodes, eight with anesthetics, one with electrolyte imbalance, one with methadone, one with procainamide, and one with an antihistamine.

Nine patients treated with antiarrhythmic drugs (e.g. propafenone, flecainide, procainamide) developed a Brugada-type ECG. These medications had been prescribed to all of the patients to prevent AF episodes. Shortly after the appearance of the Brugada-type ECG pattern, two patients experienced life-threatening arrhythmias -- one had documented VT and the other had SCD. Genetic analysis was performed in the 26 study subjects, and four of them were found to have a mutation in the SCN5A gene (Table 7).

Table 7. Inducers of Brugada-type ECG and symptoms.

<table>
<thead>
<tr>
<th>Inducer of Brugada ECG</th>
<th>Number of patients</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propofol</td>
<td>7</td>
<td>SCD: 5, VT: 1</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>2</td>
<td>SCD: 2</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>1</td>
<td>SCD: 1</td>
</tr>
<tr>
<td>Cocaine</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>Tricyclic antidepressant</td>
<td>3</td>
<td>None</td>
</tr>
<tr>
<td>Electrolyte imbalance</td>
<td>5</td>
<td>SCD: 1</td>
</tr>
<tr>
<td>Fever</td>
<td>16</td>
<td>SCD: 4, VF: 2, VT 1, syncope: 3</td>
</tr>
<tr>
<td>Methadone</td>
<td>1</td>
<td>SCD: 1</td>
</tr>
<tr>
<td>Antihistamine</td>
<td>1</td>
<td>SCD: 1</td>
</tr>
<tr>
<td>Flecainide</td>
<td>5</td>
<td>None</td>
</tr>
<tr>
<td>Procainamide</td>
<td>1</td>
<td>SCD: 1</td>
</tr>
<tr>
<td>Propafenone</td>
<td>3</td>
<td>VT: 1</td>
</tr>
</tbody>
</table>
7 Discussion

7.1 Brugada type ECG in Finland (I)

The data in I study indicate that the saddleback-type Brugada ECG pattern is not a rare observation among asymptomatic subjects. The excellent prognosis of the subjects with this ECG abnormality and the absence of any fatal or near-fatal arrhythmias during the long-term follow-up suggests that in asymptomatic subjects without a family history of SCD, type 2 or 3 Brugada ECG pattern is a normal variant rather than a specific predictor of life-threatening ventricular arrhythmias.

7.1.1 Prevalence of Brugada ECG

Although several studies have evaluated the prevalence of the Brugada type ECG in Southeast Asian populations, only scant information is available from Europe (Miyasaka et al. 2001, Matsuo et al. 2001, Furuhashi et al. 2001, Hermida et al. 2000, Atarashi et al. 2001). Given the fact that the Brugada syndrome is 8–9 times more common in men than in women, we decided to study the prevalence of the Brugada type ECG primarily from young Finnish Air Force applicants (Antzelevitch et al. 2002, Littmann et al. 2003). In this population, 15 subjects (0.61%) fulfilled the ECG criteria of type 2 or 3 Brugada syndrome (i.e., they had J-point elevation and a saddleback-type ST-segment configuration in the right precordial leads), though none of the subjects displayed a coved-type ECG abnormality. In the other population consisting of healthy middle-aged subjects, three subjects (0.55%) fulfilled the ECG criteria of type 2 or type 3 Brugada syndrome. In a large community-based Japanese population, the prevalence of the saddleback type Brugada ECG in men was 2.14% whereas the prevalence of the coved (type 1 Brugada) ECG pattern was only 0.38% (Miyasaka et al. 2001). Thus, as expected, the type 1 Brugada ECG appeared to be more common among Japanese than Finnish men. Much less data is available from other European populations.
7.1.2 Prognostic role of Brugada ECG

It is believed that the Brugada syndrome is responsible for 4–12% of all sudden deaths and about 20% of sudden deaths of patients with a structurally normal heart but major geographical differences occur (Antzelevitch et al. 2002). The prognosis of subjects with the Brugada type ECG but no history of syncope or malignant arrhythmias remains a matter of considerable debate. The results of earlier studies suggested that asymptomatic subjects have an 8–38% annual risk of experiencing life-threatening ventricular arrhythmias and sudden cardiac death (Brugada et al. 2002, Nademanee et al. 2002). Since many of the subjects were identified during routine screening after an episode of SCD in a family member, these results may not be applicable to more general populations. In the current study, none of the subjects of either study populations with the Brugada ECG (saddleback-type) died or had life-threatening ventricular arrhythmias during a relatively long follow-up period. Likewise, there were no cases of SCD among the family members of subjects with the Brugada type ECG. These findings indicate that type 2 or 3 Brugada ECG in asymptomatic individuals without a family history of SCD is a benign phenomenon, at least in these two Finnish populations. The results of several other studies in the general population, albeit with a shorter follow-up, support our data (Miyasaka et al. 2001, Matsuo et al. 2001, Furuhashi et al. 2001, Atarashi et al. 2001, Hermida et al. 2000). For example, there were no significant differences in the survival of subjects with and without the type 2 or 3 Brugada type ECG in a large Japanese study examining over 13,000 subjects (Miyasaka et al. 2001).

7.2 Saddleback type ST-segment elevation (type 2 or 3 Brugada ECG) is associated with familial lone atrial fibrillation (II)

In study II, the prevalence of saddleback-type ST-segment elevation (types 2 and 3) was found to be significantly higher among patients with lone AF than in healthy control subjects. Many patients with saddleback-type ST-segment elevation or type 2 or 3 Brugada ECG abnormalities exhibited familial clustering of lone AF. These findings suggest that there may be a link between the electrophysiological mechanism(s) underlying saddleback-type ST-segment elevation and lone AF and that genetic factors may be involved in the pathogenesis of AF in these patients.
7.2.1 Saddleback type ST-segment elevation and lone atrial fibrillation

Confirming our working hypothesis, we found that the prevalence of saddleback-type ST-segment elevation was significantly higher among the patients with lone AF than in the healthy control subjects. Despite the similar ECG repolarization abnormalities, several findings indicate that the patients described here do not have the classic Brugada syndrome. According to a recently published consensus report, only type 1 ST-segment elevation (i.e. the coved type) is diagnostic for Brugada syndrome (Antzelevitch et al. 2005a). Here, all of the patients had either type 2 or type 3 (i.e. saddleback type) ECG, both at baseline and after drug challenge. In contrast to the patients with the Brugada syndrome, none of our patients with lone AF had a personal or family history of syncope, life-threatening VT, and SCD, although Brugada syndrome patients with atrial arrhythmias have been reported to have a higher risk of VT than those without atrial arrhythmias (Itoh et al. 2001, Eckardt et al. 2001, Bordachar et al. 2004). Hence, despite the potential link between the electrophysiological features that cause the Brugada-type ECG pattern and lone AF, it is concluded that the familiar AF described here is a disease entity distinct from the Brugada syndrome.

7.2.2 Familial clustering of lone atrial fibrillation among subjects with saddleback type ST-segment elevation

The number of first-degree family members with AF was higher among the subjects with the saddleback-type ST-segment elevation. In particular, those subjects with the benign Brugada-type ECG pattern had a clear family history (i.e. AF in > 30% of their first-degree relatives). Thus, it is possible that a hereditary ion channel dysfunction may be involved in the pathogenesis of AF in these patients. The occurrence of lone AF in >30% of the first-degree relatives of the patients, i.e. familiar clustering of AF, is consistent with an autosomal dominant mode of inheritance with a high penetrance. Several population-based studies have recently described the familial aggregation of AF and a high risk of lone AF in individuals with parental AF (Ellinor et al. 2005, Arnar et al. 2006, Fox et al. 2004). In contrast to the previous studies, in our study, familial clustering of AF was associated with a distinct ECG abnormality. It is anticipated that the ongoing genetic analyses will provide additional clarification to our understanding of the genetics of lone AF.
7.2.3 Potential link between lone atrial fibrillation and saddleback type ST-segment elevation

Our data indicate that there may be a link between the electrophysiological features that cause the saddleback-type ST-segment elevation and lone AF. The familial clustering of lone AF is consistent with an autosomal dominant mode of inheritance with high penetrance. This and the close relationship between saddleback-type ST-segment elevation and lone AF support the concept that the disorder is caused by a mutation in some of the ion channels, which play a role both in the pathogenesis of AF and in the early ventricular repolarization. The ECG pattern in our patients mimicked that seen in patients with the Brugada syndrome and those with SCN5A mutations, but our patients had a more benign clinical characteristics. Mutations in the SCN5A gene were excluded in the genetic analysis. The other potential candidates include mutation(s) or a polymorphism in the genes that encode or regulate the function of the ion channels, which are active during the depolarization and early repolarization of the myocardial cells (e.g. INa, Ito, and IKur). An abnormal function of these channels is plausible since these channels are involved in the ECG pattern and they have also been shown to play an important role in the pathogenesis of AF by modulating atrial conduction velocity and refactoriness. Conduction slowing would also have a major effect on generating AF. (Nattel 2002) Regional conduction slowing in the atrial muscle could also provide a similar heterogeneous substrate to AF. Further genetic and electrophysiological studies may provide an explanation for the link between the lone AF and the saddleback ST-elevation.

On the other hand, it is tempting to speculate that the ECG changes may not have been the cause of AF, but rather its consequence. It is well established that AF begets AF by inducing electrical and structural changes in the atria and ventricles (Allessie 1998). At the cellular level, electrical remodelling is linked to downregulation of several ionic currents (e.g. Ito, IKur, ICa,L, and INa) (Bosch et al. 2002, Van Wagoner et al. 1997). At first, this seems to be incompatible with the ionic changes that initiate the saddleback-type ST-segment elevation (Antzelevitch 2001, Antzelevitch et al. 2005b). One possible explanation for this paradoxical phenomenon is that the contribution of Ito and IKur to early myocardial repolarization becomes accentuated in the remodeled tissues. Nevertheless, we found no correlation between the occurrence of saddleback-type ST-segment elevation and the duration of AF history or the total number of symptomatic AF episodes.
7.3 Clinical relevance of genetic and ECG variation in Brugada syndrome (III-IV)

7.3.1 QRS duration and vulnerability to arrhythmias

The only standard 12-lead ECG measure that differed between the asymptomatic and symptomatic patients in this relatively large Brugada syndrome patient cohort was the duration of the QRS complex. Other ECG measures did not achieve statistical significance in this respect.

Basically, Brugada syndrome is characterized by abnormal repolarization, which has been shown to increase the arrhythmia vulnerability in experimental models (Kimura et al. 2004). In addition to altered repolarization, the prolonged QRS duration in Brugada syndrome has been previously described and mentioned as one of the features related to the syndrome (Smits et al. 2002, Antzelevitch et al. 2005a). Thus, Brugada syndrome is also associated with abnormal depolarization and impulse conduction. The present data show that this conduction abnormality may also be important in the genesis of clinical arrhythmias in the disease. This finding concurs with those of Aiba et al. in experimental Brugada syndrome models, where the depolarization abnormalities have been associated with factors that predispose to VF maintenance (Aiba et al. 2006).

The reasons why prolonged QRS duration is associated with symptoms and vulnerability to ventricular tachyarrhythmias in Brugada syndrome have been studied previously in an experimental setting (Aiba et al. 2006). Decreased I_Na current causes the typical Brugada sign in the 12-lead ECG with the aid of normal I_To current. In approximately 20% of Brugada syndrome subjects, the decline in the sodium current can be traced to a mutation in the SCN5A gene that encodes the pore-forming region of the sodium channel, but in the remainder of subjects the genetic background is still unknown. It is possible that a more marked abnormality in the I_Na current results in prolonged depolarization, in addition to a repolarization abnormality, and thereby increases the risk of arrhythmias. In some cases, the QRS prolongation could be explained by the SCN5A mutation since the SCN5A mutation carriers had a trend towards longer QRS in lead V2 compared to the Brugada syndrome subjects without SCN5A mutation, in agreement with finding reported by Smits et al (Smits et al. 2002).
7.3.2 Risk stratification of Brugada syndrome patients

The clinical risk assessment of Brugada syndrome has largely relied on prior symptoms and VT/VF inducibility in an EP study (Brugada et al. 2003, Priori et al. 2002, Gehi et al. 2006, Eckardt et al. 2005, Glatter et al. 2005, Antzelevitch et al. 2005a). The so-called symptomatic patients or subjects with prior aborted SCD, syncope or documented ventricular arrhythmia, are likely to have symptom recurrence and thus often need an ICD implantation to prevent SCD. The major problem lies in the asymptomatic subjects, and in trying to determine who will remain asymptomatic. Brugada et al. have described that EP study is an effective risk assessment tool in these patients, but Eckardt et al. have stated that EP study does not help in SCD prediction in asymptomatic subjects (Brugada et al. 2003, Eckardt et al. 2005). Many controversies remain to be resolved around this issue.

Risk markers of symptoms in Brugada syndrome have also previously been sought from the standard ECG. The amplitude of J-point elevation in symptomatic subjects versus asymptomatic has been shown to be higher (Eckardt et al. 2005, Ikeda et al. 2001). In our study, the J-point elevation in none of the right precordial leads V1-3 correlated with prior symptoms.

7.3.3 Genetic modulators in Brugada syndrome

In this study we showed that Brugada syndrome subjects with SCN5A mutation and the wild type AA genotype in variant H558R have longer QRS duration, more impaired repolarization characteristics (reflected in higher J-point elevation in right precordial leads) and a higher “aVR sign”. The G allele carriers had a trend towards less symptoms compared to AA genotype carriers.

7.3.4 H558R effect in sodium channel function

Previously the H558R variant G allele has been associated with the occurrence of early onset AF and a longer QTc interval in healthy populations (Chen et al. 2007, Gouas et al. 2005). The variant H558R G allele frequency has been reported to be 20.4% in white population, 29% in black population and 10.4% in the Chinese population (Ackerman et al. 2004, Chen et al. 2004). In our study of a mostly white population, the G allele frequency was 31% of SCN5A mutation carriers and 16% in non-SCN5A mutation patients. A higher frequency of allele G in Brugada syndrome subjects with SCN5A mutation than without was also
observed among Chinese Brugada syndrome patients (Chen et al. 2004). According to these findings, one might speculate that the H558R G allele may be protective and allow the carrier to reproduce and transfer the mutation and the variant in their family because they have less symptoms and less chance of suffering SCD. On the other hand, patients without the protecting G allele could have a higher chance of dying suddenly before they progenerate causing the mutation to perish with them.

Earlier in vitro studies and one case of a salvaged SCN5A mutation carrier have shown that the H558R variant G allele restores sodium channel function in Brugada syndrome subjects with SCN5A mutation even when it is present in the opposite allele to the mutation (Viswanathan et al. 2003, Ye et al. 2003, Poelzing et al. 2006). These findings have also been confirmed in the clinical portion of this study. The mutation carriers who also have the less common allele G have improved ECG characteristics and seem to have fewer symptoms than subjects with the AA genotype. However, the role of H558R as a genetic modulator appears to be gene specific as it does not cover the non-SCN5A mutation Brugada syndrome subjects, since none of the ECG parameters or symptoms displayed differences between genotype groups in the non-SCN5A mutation Brugada syndrome subjects in our study.

7.4 Induced Brugada ECG in acute medical conditions (V)

This study provides data on 47 patients with a Brugada-type ECG induced by several factors known to unmask the Brugada syndrome. From our 47 subjects, 24 had developed symptoms related to the Brugada syndrome during the acute event, including 18 patients with SCD, three with VT, and three with syncope. Based on our results, the presence of the Brugada ECG pattern in patients during an acute event such as fever, treatment with sodium channel affecting medication, drug overdose, or electrolyte imbalances should be considered a risk factor for the development of life-threatening cardiac arrhythmias.

Several fever-induced Brugada-type ECG cases have been previously described in the literature. Studies have reported mutant sodium channels that exhibit temperature-dependent gating changes consistent with more evident ECG abnormalities at increased temperature (Porres et al. 2002, Dumaine et al. 1999). Regardless of the existence of a predisposing genetic base, most of our patients with fever and a Brugada-type ECG developed malignant arrhythmias shortly after the onset of the fever.
A number of substances facilitate the elevation of the ST segment either by reducing the inward sodium current or by increasing the outward potassium current (Vernooy et al. 2006b, Aksay et al. 2005, Bebarta et al. 2007, Brugada et al. 2006). Sodium channel blockers, cocaine, antidepressants, and antihistamines are known to facilitate the Brugada-type ECG by reducing the inward current. Hyperkalemia, vagotonic agents, and IKATP activators augment the outward current, which is also transduced in the ECG as the typical Brugada pattern. In our study, a total of 19 patients had received substances known to reduce the cardiac sodium current. All patients were prescribed antiarrhythmic medication to prevent AF and two of these patients’ experienced life-threatening arrhythmias (VT and SCD) while taking the medication. Therefore, we conclude that patients who are treated with class Ic medication and who present with a Brugada-type ECG are at high risk of suffering ventricular arrhythmias.

Anesthetics such as bupivacaine, lidocaine and propofol are commonly used in present daily clinical practice. Genetic mutations in the SCN5A gene have been found in patients with a bupivacaine-induced Brugada-type ECG (Vernooy et al. 2006b). Similarly, propofol at high dosages can contribute to the development of lethal arrhythmias (Vernooy et al. 2006a). The term Propofol Induced Syndrome (PRIS) has been used to describe its fatal outcome. Consistent with these findings, two of our patients who had received bupivacaine did develop arrhythmias shortly after its administration. In this study, we had seven subjects with a propofol infusion and a Brugada-type ECG; five of these patients had experienced SCD and one patient developed VT. Therefore, we conclude that the presence of a Brugada-type ECG during the infusion of bupivacaine, lidocaine or propofol is an indicator of imminent malignant arrhythmias. Bupivacaine and lidocaine are known to be sodium channel blockers. The mechanism by which propofol unmasks this ECG pattern is not yet clear. Many experimental studies have been conducted to elucidate the ECG alternations evoked by anesthetics and propofol but these compounds do not appear to cause significant changes in baseline electrophysiology in the standard situation. Therefore, one can speculate that the subjects in this study might have an underlying, possibly genetic, condition that increases the electrophysiological effects of propofol. (Napolitano et al. 1996, Morey et al. 1997, Raatikainen et al. 1998)
7.5 Methodological considerations and limitations

The heterogeneity of the study populations is large. There are major differences in Finnish populations (I-II) and worldwide Brugada syndrome populations (III-V) and thus comparisons with these studies are not possible. The clinical impact of the findings in studies III-V might have greater international impact; in Finland these findings might not have great practical significance in health care due to relative rarity of Brugada syndrome cases. Additionally, in cohorts of such a rare disease as Brugada syndrome or even LQT syndrome selection bias occur when physicians tend to send DNA samples or clinical data of patients with adverse events to the international databases. Thus, it is almost certain that studies conducted with the population of these databases include patients with somewhat unstable form of the disease and could give biased results.

Another limitation in this thesis is the case-control nature of these crossectional studies II-IV. Therefore, generalization of the results is not possible. Prospective follow-up studies would give more reliable information for risk stratification in this respect. However, in studies III-IV, it was known that Brugada syndrome subjects with prior symptoms are predisposed to symptom recurrence (Brugada et al. 2002, Brugada et al. 2003, Eckardt et al. 2005, Priori et al.a 2002, Gehi et al. 2006, Glatter et al. 2005) and thus these ECG and genetic characteristics related to symptomatic subjects also provide indirect information on risk of symptom occurrence.

Most physicians find the interpretation of the ECG to be a very difficult task. For example, the inter-observer measurement variability in long QT patients have been found to be very large (Viskin et al. 2005). In Brugada syndrome the accuracy of ECG diagnosis, not to mention the prognostic evaluation, might be an even greater problem. In studies III-IV, the QRS duration was measured and found to be significantly different between symptomatic and asymptomatic subjects. This finding raises the question of QRS interval interpretation in the presence of a J-wave which causes obvious difficulties. Therefore, we determined the J-point as a consensus from other precordial leads which do not present a J-wave in Brugada syndrome and we also evaluated the QRS measurement from lead II. Although these actions were taken to diminish possibility of false results, the reproducibility of the results even in the same population might be difficult even though a study with similar results has recently been published (Takagi et al. 2007).
8 Conclusions

1. The results of the present study suggest that type 2 or 3 Brugada ECG pattern can be observed among healthy subjects without a risk for arrhythmias or structural heart disease and it can be considered as a normal variant with respect to the Brugada syndrome diagnosis.

2. Saddleback-type ST-segment elevation (type 2 or 3 Brugada ECG) is significantly more frequent among patients with lone AF than healthy control subjects. The familial clustering of the disorder indicates that a hereditary ion channel dysfunction may be involved in the pathogenesis of ECG abnormalities and lone AF in these patients. Further genetic analyses are needed to reveal the molecular mechanism behind this disorder.

3. Prolonged QRS duration, measured from the standard 12-lead ECG, is associated with symptoms and could become a useful additional non-invasive risk marker of life-threatening ventricular arrhythmias in Brugada syndrome. Whether the prolonged QRS duration is a marker of future arrhythmia events in asymptomatic patients will be examined in a follow-up study in the imminent future.

4. The common variant H558R seems to be a genetic modulator of Brugada syndrome among carriers of a SCN5A mutation in whom the presence of the less common allele G improves the ECG characteristics and clinical phenotype.

5. Patients who suffer from acute medical conditions and who present with a Brugada-type ECG are at a considerably higher risk of SCD and should be considered as a medical emergency. A therapeutic intervention with antipyretics, or by stopping the culprit medication, is warranted. These data also indicate that individuals with Brugada syndrome and their family members who are genetic carriers of a sodium channelopathy should be aware that some medications and disease states may increase their risk of arrhythmias.
References


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**Original articles**

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals:


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Original publications are not included in the electronic version of the dissertation.
950. Anttonen, Vuokko (2007) Laser fluorescence in detecting and monitoring the progression of occlusal dental caries lesions and for screening persons with unfavourable dietary habits


955. Löfgren, Eeva (2007) Effects of epilepsy and antiepileptic medication on reproductive function


959. Papponen, Hinni (2008) The muscle specific chloride channel ClC-1 and myotonia congenita in Northern Finland


962. Dunder, Teija (2008) Environment and atopy and asthma in childhood. The effect of dietary fats, common infections and asthma treatment practises on morbidity rates


964. Rysä, Jaana (2008) Gene expression profiling in experimental models of cardiac load
Juhani Junttila

CHARACTERISTICS OF SUBJECTS WITH BRUGADA SYNDROME TYPE ELECTROCARDIOGRAM