# **Familial form of sick sinus syndrome.** New views on polygenic origin and prospects for gene therapy

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**Absract**

This review article presents the new data on the polygenic origin of the familial (congenital) form of sick sinus syndrome (SSS) and the approaches to gene therapy of this pathology. We also provide information concerning the epidemiology of SSS and the acquired (secondary) causes of SSS. The article presents detailed characteristics of the main genes associated with the development of SSS and the mechanisms of ion channel disorders responsible for arrhythmogenesis. Genetic and phenotypic variants associated with the development and clinical course of familial SSS are also described. We also present the latest achievements in the field of SSS gene therapy and prospects for its development.

**Key words**: sick sinus syndrome, sudden cardiac death, cardiac arrhythmias, pacemaker, gene therapy.

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## Introduction

Idiopathic or congenital (familial) sick sinus syndrome (SSS) is a primary arrhythmogenic (electrical) disease, genetically heterogeneous and associated with the risk of sudden cardiac death (SCD) [1–3]. In 2003, a familial form of SSS with autosomal recessive type of inheritance was described. It is associated with the mutation in the SCN5A gene that results in impaired sodium channel function[4, 5]. The etiology of SSS remains unspecified, and its clinical manifestations are nonspecific, which complicates its diagnosis and adequate treatment [6, 7]. Given the low survival rate (less than 10 %), the identification of patients with high-risk SSS is essential for the prevention of SCD [8].

It should be noted that modern scientific and medical literature often uses different definitions to describe this condition. According to the current version of ICD-11, the term "sinus node dysfunction" is a broader definition than SSS. Usually, only ECG signs of sinus node (SN) damage without clinical symptoms and/or absence of structural heart lesions are referred to as sinus node dysfunction [6, 9].

## SSS Epidemiology

Although SSS is more common in the elderly and in people with structural heart disease, its true prevalence in the general population is unknown. According to the available incomplete information, it is approximately 3:5000 in cardiology patients [2], and according to other data, SSS occurs in 1 of 600 patients with heart disease older than 65 years [10, 11]. There have been reports of SSS signs in 6.3–24 % of the patients with electrocardiostimulators (ECS) [12]. According to some data, this syndrome is an indication for ECS implantation in 30–50 % of cases in Europe and USA [13]. SSS is considered to be equally common in men and women. It has been also shown that in patients who come to the clinic for the first time due to heart rhythm disturbances (HRD), SSS is detected in 3 % of cases, and among those suffering from syncope of unclear etiology, this syndrome occurs in every third person [2].

Jensen P.N. et al. (2014) in the prospective study with average 17-yearfollow-up of 20572 participants identified SSS in 291 patients. Incidence increased with age: over 5 years, the relative risk (RR) was 1.73 [95 % confidence interval (CI) 1.47–2.05]; black patients had 41 % lower risk of SSS than white ones (RR, 0.59; 95 % CI: 0.37 to 0.98). The authors prognose an increase in the annual number of new cases of SSS in the United States from 78,000 in 2012 to 172,000 in 2060. The overall annual incidence of SSS in individuals aged 45 years and older has been shown to be close to 1 per 1000. The age- and race-standardized rate of SSS was 0.8 per 1,000 person-years in women and 0.9 per 1,000 person-years in men [14].

## Clinical presentation of SSS

Despite the genetic determinism of familial SSS, the manifestation of symptoms, its severity and prognosis, as well as the choice of therapeutic strategies are determined by acquired diseases [4, 6, 9]. Secondary SSS is caused by external (exogenous) factors (pharmacological, metabolic or autonomic) that have a depressing effect on SN function: hyperkalemia, hypercalcemia, treatment with certain medications that reduce SN automatism, etc. [6, 10]. Moreover, SSS is often a remote complication of cardiac surgery [2].

In most cases SSS is asymptomatic [15]. It is characterized by persistent and inadequate sinus bradycardia, episodes of sinoatrial block and/or chronotropic incompetence [1]. Alternating bradycardia and tachycardia, i.e. tachycardia-bradycardia syndrome, is observed in at least 50 % of patients with SSS [16, 17]. Despite great efforts to study the mechanisms of SSS in terms of abnormal automatism, blockade of SN "output" or intraatrial conduction and excitability disorders, this diagnosis remains mainly electrocardiographic [1, 2, 15].

SSS causes bradyarrhythmia with wide clinical spectrum from no symptoms to SCD [7]. Clinical symptoms result from hypoperfusion of target organs [1]. Two main groups of symptoms form the clinical presentation of SSS: cardiac and cerebral. About 50 % of patients have signs of cerebral hypoperfusion: dizziness, fainting, impaired cerebral circulation. During exercise, many patients experience chest pain, shortness of breath, difficulty breathing or excessive fatigue. This condition increases the risk of life-threatening cardiac events: HRD, atrial fibrillation (AF), heart failure, cardiac arrest and stroke [10].

Fainting may be triggered by coughing and abrupt change in head position, as well as by standing up quickly and being in an unventilated room that is caused byvagotonia. Cardiac syncope is characterized by the absence of aura, seizures (excluding cases of prolonged asystole) and usually has short duration

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and stops on its own, but if prolonged may require resuscitation [4].

Elderly patients may have impaired memory and intellect. Progression of bradycardia may be accompanied by the phenomena of discirculatory encephalopathy (appearance or increase in dizziness, momentary lapses in memory, paresis, irritability, insomnia). Due to hypoperfusion of internal organs, oliguria, acute ulcers of the gastrointestinal tract may develop, the symptoms of intermittent claudication and muscle weakness may increase. Cold and pale skin with a severe drop in BP and cold sweats are possible.

SSS also has stages in its course. Usually starting with one of the initial forms, it gradually progresses to an advanced form of SSS [6, 15]. However, there is often a wave-like course of SSS, when patients with initial manifestations of the syndrome are either detected or disappear. Such fluctuations are caused by different reasons — due to the course of the underlying disease, the dynamics of autonomic effects, medications, etc. Depending on the disease features, clinicians distinguish latent, intermittent and manifesting SSS [1]. Binodal disease is considered a variant of severe SSS, which manifests with a combination of SSS and atrioventricular block (AVB).

Depending on the clinical manifestation, the following forms of SSS and their courses are distinguished [1, 4, 6]:

1) Latent form — absence of clinical and electrocardiographic manifestations. SSS is proved by electrophysiological study.

2) Compensated form: ECG manifestations of SSS are detected, but there are no clinical symptoms of the disease.

3) Decompensated form: SSS is manifested by clinical and ECG signs, thus, there is a clear correlation between the severity of clinical symptoms and the severity of bradycardia. Syncope, decreased tolerance to physical activity, development of heart failure, etc. are possible, which are often indications for ECS implantation.

4) Permanent form of AF with previously diagnosed SSS. It often manifests by tachycardic form of AF, requiring drug-assisted HR control, and later as tachycardic cardiomyopathy (CMP) with the signs of heart failure. The CMP may progress to a persistent bradycardic AF with cardiac pauses and (or) Morgany-Edems-Stokes attacks, requiring ECS implantation.

## Genetic etiology of SSS

The literature mentions familial clustering of SSS. Both autosomal recessive and autosomal dominant forms have been described [5, 18, 19]. Currently, 22 genes are associated with the development of hereditary SSS [3]. Molecular genetic studies have confirmed that SSS can be caused by mutations in certain genes (Table 1) [5].

### *CASQ2 and RYR2 receptors*

Cardiac calasequestrin-2 (Casq2), localized in the sarcoplasmic reticulum (SR), is a low-affinity and high capacity $Ca^{2+}$-binding protein, which regulates the ability of SR to depot and release $Ca^{2+}$ in cardiomyocytes [20]. Potential-dependent L-type calcium channels create an initial influx of $Ca^{2+}$ into the cell, which causes RyR2 receptors to release more calcium from the SR in a process known as calcium-induced calcium release. Calsequestrin, the major $Ca^{2+}$-binding protein in the SR, attaches to the membrane of the SR via RyR2 (directly or indirectly via triadin and junctin) and regulates $Ca^{2+}$ release via the RyR2 channel. It is important to note that calsequestrin, the ryanodine receptor, junctin, and triadin are essential for the normal calcium cycle in myocytes.

Variants with loss of CASQ2 function can lead to catecholaminergic polymorphic ventricular tachycardia (CPVT), bradycardia, and atrial arrhythmias [21]. It has been shown that CPVT is also induced by RYR2 variants and is closely associated with bradycardia [22]. Thus, the detection of SSS and atrial arrhythmias in patients with CPVT indicates the importance of CASQ2 and RYR2 receptors for normal SC functioning.

### *G protein-coupled inwardly-rectifying potassium channel 4 (KCNJ5)*

G-protein activation via a transmitter is a common type of intercellular communication. Typically, the neurotransmitter binds to the seven-membrane receptor outside the cell, resulting in the exchange of GDP for GTP on the inner side of the receptor, which allows the dissociation of heterotrimeric G-protein subunits, which then act as effectors [23].

To modulate heart rate, the Gbg subunit specifically activates the muscarinic acetylcholine potassium channel (KAch) by binding directly to the N- and C-termini of GIRK1 (G protein-coupled inwardly-rectifying potassium channel) and GIRK4 [5]. The

*Table 1.* **Genes and proteins involved in the development of SSS in humans**

| Protein | Genes | Associated cardiac diseases |
|---|---|---|
| Calsequestrin-2 | CASQ2 | SSS/bradycardia, CPVT, atrial arrhythmias |
| Ryanodine-2 Receptor | RYR2 | SSS/bradycardia, CPVT, atrial arrhythmias |
| G protein-coupled inwardly-rectifying potassium channel 4 (KCNJ5) | KCNJ5 | SSS/bradycardia, atrial arrhythmias, Long QT syndrome type 13, Andersen–Tawil syndrome |
| Signal-Transducing Guanine Nucleotide-Binding Regulatory Protein Beta Subunit 2/5 | GNB2/GNB5 | SSS/bradycardia, cognitive impairment, cardiac conduction disorders |
| Sodium channel protein type 5 subunit alpha | SCN5A | SSS/bradycardia, Long QT syndrome type 3, Brugada syndrome, dilated cardiomyopathy, conduction disorders, sudden infant death syndrome |
| Sodium/calcium exchanger-1 | SLC8A1 | Conduction disorders, ventricular arrhythmias, Kawasaki disease |
| Potassium hyperpolarization-activated cyclic nucleotide-gated channel 4 | HCN4 | SSS/bradycardia, ventricular arrhythmias, Noncompaction cardiomyopathy of left ventricle |
| Ankyrin-B | AHK2 | SSS/bradycardia, CPVT, atrial arrhythmias, arrhythmogenic CMP |
| Myosin Heavy Chain | MYH6 | SSS/bradycardia, coarctation of the aorta, ventricular arrhythmias |
| Lamin A | LMNA | SSS/bradycardia, dilated cardiomyopathy, conduction disorders |
| Voltage-Gated Calcium Channel Subunit Alpha Cav1.3 | CACNA1D | Sinus node dysfunction and deafness |
| Short Stature Homeobox Protein 2 | SHOX2 | SSS/bradycardia, atrial arrhythmias |

KAch channel consists of GIRK1 and GIRK4 subunits (Kir3.4, KCNJ5 gene) in the atria and contributes to heart rhythm regulation. Parasympathetic stimulation activates KAch channels, resulting in slowing heart rate and decreasing cardiac contractility [24]. In mice with Girk4 knockout, not only potassium current through IKAch channel but also Girk1 expression were absent, which confirms the fact that Girk4 plays a leading role in the expression and localization of Girk1 on the cell membrane. The KAch channel is rapidly and reversibly inhibited by membrane stretch, making atrial mechanoelectric regulation possible.

### *Signal-Transducing Guanine Nucleotide-Binding Regulatory Protein Beta Subunit 2/5*

Notably, subunits 2 and 5 of the guanine nucleotide-binding proteins (GNB2 and GNB5), which create the beta-subunits of G-protein that interact with GIRK1 and GIRK4, also play a role in the development of SSS. GNB5 variants were reported in patients with sinus bradycardia and cognitive impairment. The GNB2 variant has been more closely associated with cardiac conduction abnormalities — SSS and AVB [25].

In 2019, the GIRK4 variant (W1010C) was first identified in a three-generation family with SSS. Interestingly, the W1010C variant in GIRK4 resulted in an increase in IKAch. This variant of enhanced channel function caused increased parasympathetic tone, causing a familial variant of SSS and hyperpolarization of SN cells [23].

### *Sodium channel protein type 5 subunit alpha*

Recent mutation analysis reports have identified more than 200 different mutations in SCN5A, of which at least 20 mutations are associated with SSS [3]. In addition to variable expression, heterozygous SCN5A mutations have shown an incomplete penetrance. Complex heterozygous SCN5A mutations are associated with a recessive form of congenital SSS [26].

Depolarization of atria, ventricles, and Purkinje myocytes, which causes cardiac contraction, is initially regulated by the Nav1.5 sodium channel (pore-forming, ion-conducting **α**-subunit of the heart sodium channel) encoded by SCN5A. Variants of this gene are involved in a wide range of cardiac diseases, such as Brugada syndrome, congenital long QT syndrome (LQTS), AF, SSS, dilated cardiomyopathy (DCM), etc. [27, 28]. SCN5A variants (usually of the autosomal recessive type) have been associated with SSS and conduction abnormalities in humans [15].

Because SCN5A is not expressed in SN and SN action potentials are independent of SCN5A, primary SSS seems unlikely. However, given that the inability of pulses to conduct into the atrium due to the "exit" blockade has been proposed as a cause of SSS [2], this is a likely explanation for the genesis of SCN5A-associated SSS. In addition, certain genetic variants of connexin-40 (Cx40) with concomitant SCN5A mutations have been shown to result in the atrial arrest phenotype [26].

The SSS phenotype identified in SCN5A variants is usually secondary to Brugada syndrome and LQTS3.

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Thus, it has been shown that out of 41 people with the identified E1784K loss-of-function variant, 39 % had SSS, and almost all (93 %) had LQTS3 [28].

### *Potassium hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4)*

Mutations have also been found in the HCN4 gene encoding the α-subunit of a hyperpolarization-activated, ATP-dependent cation channel, predominantly expressed in the SN, responsible for the pacemaker current — If current [28]. This leads to a decrease in pacemaker current, a consequence of which is a severe sinus bradycardia, in some cases combined with prolongation of QT interval and ventricular tachycardia (VT) of pirouette type.

The ability of pacemaker cells of SN to spontaneously initiate an electrical impulse can be explained by the activation of If current, the current of Na+/K+ depolarization [29]. If is the incoming current during diastolic depolarization (phase 4 of Action Potential (AP)), which is then activated by membrane hyperpolarization (hypothesis of "membrane clock" for SN automatism) through binding to intracellular cAMP, which can be modified by sympathetic and parasympathetic pulses, and thereby modulate HR [30]. If-current can also be regulated by cAMP-activated protein kinase A in the SN [29].

Many studies have shown that of the four members of the HCN channel family, HCN2- and HCN4-based channels are expressed in the SN [31]. Moreover, the HCN4 channel has a higher level of expression, which confirms the leading role of HCN4 in controlling the pacing activity of the SN. HCN4 channels and ß2-adrenergic receptors form a complex required for HCN4 channel regulation [30]. Interestingly, the expression of HCN2 and HCN4 channels has been shown to decrease in SN and increase in the atria and pulmonary veins with age, which may explain the disproportionately higher incidence of SSS in the elderly [11].

HCN4 encodes a Potassium hyperpolarization-activated cyclic nucleotide-gated channel 4, which contributes to the native currents of the pacemaker cells of the SN — If current [31]. More than 150 variants of HCN4 have now been reported [30]. Mutations in HCN4 may cause sporadic and familial forms of SSS [18]. In addition to bradycardia, pharmacological blockade of If and genetic knockdown of HCN4 caused long cardiac pauses [32].

The HCN4 variant (573X) causing HCN4 C-terminal shortening was first identified in a patient with SSS manifesting sinus bradycardia and chronotropic incompetence. In addition, familial sinus bradycardia is associated with C-terminal shortening and loss of cAMP-dependent regulation of HCN4 [29]. The G482R HCN4 variant has been reported in a family with bradycardia and noncompact LV [33]. It has also been shown that 573X HCN4 variant causes suppression of If channel sensitivity to cAMP, decreases maximal rate of pacing cell depolarization of SN and HR both at rest and during exercise. This demonstrates that cAMP-mediated regulation of the If current determines baseline and maximal HR, but is not involved in HR adaptation during physical activity [32].

### *Sodium/calcium exchanger-1 Precursor (SLC8A1)*

The sodium-calcium exchanger (NCX) is a cytoplasmic membrane transmembrane protein that transports $Ca^{2+}$ from the cell in exchange for $Na^{+}$, which enters the cell (via anti-port mechanism). To do this, the exchanger uses the energy stored in the electrochemical sodium gradient, passing three sodium ions into the cell along the concentration gradient and removing one calcium ion from the cell against the concentration gradient.

The cardiac $Na^{+}/Ca^{2+}$ exchanger (NCX1) plays an integral role in diastolic depolarization, which triggers these recurrent APs [34]. After diastolic $Ca^{2+}$ release from the SR via ryanodine receptors, increased cytosolic $Ca^{2+}$ induces an incoming current through NCX, which accelerates late diastolic depolarization to the AP threshold (a "calcium clock" model for pacemaker cell automatism). In addition, inactivation of NCX1 causes complete cessation of SN activation by generating intermittent pulse activation due to intracellular $Ca^{2+}$ overload [35]. NCX1 has 10 transmembrane helices and four ion-binding sites, one for calcium ions and three for sodium ions [36].

NCX1 functions by providing both incoming and outgoing currents depending on the membrane potential. NCX1 is the main way to extrude ("squeeze out") calcium from cardiomyocytes during the resting membrane potential; the high extracellular sodium concentration allows NCX1 to remove calcium from the cell [35]. In general, NCX promotes myocyte relaxation, indicating the role of NCX1 in the contractile process. In addition, spontaneous release of $Ca^{2+}$

from pacing cells by RyR2 activates NCX1 at the SR membrane, which then pushes the cell to the minimum threshold to trigger PD [36].

Genetic variants in SLC8A1, which encodes NCX1, cause changes in the calcium cycle, leading to ECG signatures of SSS. However, no cases of correlation of SLC8A1 mutation and development of SSS in humans have been reported so far. At the same time, animal models have shown the main SSS phenotypes with loss of NCX1 function [35].

### *Ankyrin-2 (ANK2)*

Ankyrin-B (AnkB, encoded by ANK2) is a membrane adaptor protein critical for recruitment, organization, and stabilization of ion channels and transporters underlying excitation and contraction coupling, particularly in SN. Variants of function loss in ANK2 are associated with a complex cardiac phenotype, including heart rate variability, CPVT, conduction defects, AF, sinus bradycardia, HF, and arrhythmogenic CMP [5].

Two families with severe SSS were found to have variants of the ANK2 allele, making AnkB the first nonionic protein associated with human SSS [37]. Interestingly, people with AF have decreased expression levels of AnkB and increased levels of miR-34a (an amiRNA associated with cardiac fibrosis). The untranslated region 30 of ANK2 also contains a binding site to miR-34a, suggesting a potential role for miR-34a in atrial electrical remodeling and in the regulation of AnkB expression [38].

Although the results of AnkB clinical studies suggest that ANK2 loss-of-function variants are associated with numerous cardiac diseases, the lack of family history in many of these cases and the overall incomplete penetrance of AnkB-associated disease strongly suggest that additional genetic and/or environmental factors must be involved in the development of the severe AnkB syndrome phenotype [18]. Notably, intense endurance exercise or other genetic variants probably play a role in the development of heart disease associated with variants of ANK2 loss of function [37].

### *Myosin Heavy Chain (MYH6)*

MYH6 encodes the α-subunit of myosin heavy chain (TCM-α), a major component of the sarcomere, a necessary component of muscle fiber for proper heart contraction [39]. The MYH6 myosin heavy chain gene is localized in the long arm of chromosome 14, locus 11.2. The missense variant of TCM-α R721W has been identified in Icelandic populations (0.38 % allelic frequency) and is associated with SSS, and 50 % of carriers of this variant diagnosed with SSS. Carriers of the variant who were not diagnosed with SSS still had a decreased HR and prolonged PR interval [38]. Interestingly, another heterozygous variant R654W of TCM-α was identified in an Australian family with severe but diverse cardiac arrhythmias, including SSS and ventricular fibrillation (VF) leading to HF [39].

### *Lamin A (LMNA)*

Lamins A, B1 and B2 are the main components of the nuclear lamina, which plays a vital structural role in the nuclear envelope [5]. LMNA variants are associated with numerous heart diseases, particularly with dilated CMP. A heterozygous c.357–2A >G splice site variant in LMNA was identified in a proband diagnosed with SSS who had a family history of cardiac arrhythmias and SSS [20]. It was hypothesized that this new variant causes haploinsufficiency because the aberrant mRNA from the mutant allele is likely to decay due to nonsense-mediated mRNA decay. Although no LMNA population variant has been identified regarding SSS, the numerous identified familial variants associated with conduction abnormalities provide a basis for further investigation of the lamin A role in the development of SSS.

## C Voltage-Gated Calcium Channel Subunit Alpha Cav1.3 (CACNA1D)

Cav1.2 (α-1C) and Cav1.3 (α-1D) subunits make up the potential-activated cardiac L-type calcium channels [3]. Cav1.3 is expressed mainly in SN, AV node and atrial myocytes [35]. In SN, Cav1.3 plays a pivotal role in pacing cell activity, controlling the incoming current during diastolic depolarization and regulating diastolic $Ca^{2+}$ release from the SR [35].

T-type calcium channels consist of three subunits, Cav3.1, Cav3.2, and Cav3.3, which are encoded by three genes: CACNA1G, CACNA1H, and CACNA1I. The three Cav subunits are responsible for the generation of T-type/low-voltage activated calcium currents (T-currents) [1]. Notably, earlier studies reported the expression of calcium current T-type ($I_{CaT}$) in SN cells and suggested its contribution to pacemaker cell function [7].

A direct contribution of Cav3.1 channels to cardiac SN automatism and conduction functions has been

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demonstrated through genetic ablation of Cav3.1 channels in mice. Genetic inactivation of Cav3.1 channels causes slowing of SN automatism by decreasing the slope of diastolic depolarization in SN pacing cells [35].

The role of other genes involved in the regulation of SN pacing cell function and phenotyping various manifestations of SSS also continues to be investigated: e.g., short stature homeobox protein 2 (SHOX2), transient receptor potential channel 3 (TRPC3), stromal interaction molecule 1 (STIM1), etc. [3–5].

## Genotype-phenotypic variants of SSS

Considering the genetic defects and phenotypic manifestations, 4 types of SSS are distinguished [3]. In most cases, SSS is not inherited [10] and is described as sporadic [2]. When SSS results from mutations in the HCN4 gene, it has an autosomal dominant type of inheritance, which means that one copy of the altered gene in each cell is enough to cause the disease [20]. In most cases, one parent of the affected individual has the disease. When SSS syndrome is caused by mutations in the SCN5A gene, it is inherited by autosomal recessive type, which means that both copies of the gene in each cell have mutations [26]. Each parent of a person with autosomal recessive disease carries one copy of the mutated gene, but usually they have no signs and symptoms of the disease.

Type 1 (autosomal recessive type SSS). Congenital SSS type 1 is caused by a compound heterozygous mutation in the SCN5A gene on chromosome 3p22. Mutation in the SCN5A gene associated with SSS type 1 causes association with the following diseases: thyroid dyshormonogenesis, familial AF, ventricular arrhythmias due to calcium release deficiency syndrome from cardiac ryanodine receptors, Brugada syndrome, cardiac conduction abnormalities with or without dilated CMP [1].

SSS type 1 most often occurs in the elderly and is associated with underlying heart disease or prior heart surgery, but can also occur in a fetus, infant, or child without heart disease or other contributing factors. ECG usually shows sinus bradycardia, SN arrest and/or sinoatrial block, prolonged QT interval, and AVB. In addition, a combination of episodes of atrial tachycardia and sinus bradycardia ("tachycardia-bradycardia syndrome") is common. Manifestation of SSS type 1 occurs in fetal state, in infancy or early childhood.

Online Mendelian Inheritance in Man (OMIM) is a medical database that collects information about known diseases with a genetic component and the genes responsible for their development. SSS type 1 is characterized by the following cardiovascular symptoms from the clinical synopsis, according to OMIM: sinus bradycardia, atrial arrest, SN arrest, sinus arrhythmia, absence of P waves, increased QRS duration, sporadic idioventricular rhythm, increased Hys-ventricular conduction time, no structural heart defects, first degree heart block or conduction delay can be observed in heterozygous mutation carriers.

Type 2 (autosomal dominant type SSS or SSS type 2 with "noncompact" LV and/or dilatation of the ascending aorta or familial AF with bradyarrhythmia) [33]. The responsible gene associated with SSS type 2 is HCN4, located in chromosome 15q24 [31]. Electrocardiographic phenotypes are sinus bradycardia, SN and/or sinoatrial arrest, and AVB. Tachycardia-bradycardia syndrome is also common. SSS occurs most often in older adults associated with underlying heart disease or prior heart surgery, but can also occur in a fetus, infant, or child without heart disease or other contributing factors. SSS type 2 begins in a prenatal period or at birth. Affected tissues include the heart, and related phenotypes are mitral valve prolapse and LV hypertrophy.

Cardiovascular symptoms of SSS type 2 that occur in the clinical synopsis (OMIM): sinus bradycardia, in some patients — LV hypertrophy, AF, VF, cardiac arrest (rare). Additionally, dilation of the ascending aorta (in some patients) may present. Concomitant cardiac phenotypes are detected in about 7.5 % of cases: mitral valve prolapse, myxomatous degeneration of the mitral valve, LV hypertrophy, biventricular hypertrabecularity, aortic regurgitation, cardiac arrest and VF.

Type 3 SSS most often occurs in the elderly with the background of underlying heart disease or prior heart surgery. At the same time, SSS type 3 can occur in a fetus, infant, or child without heart disease or other contributing factors. Symptoms are often intermittent and/or nonspecific and include dizziness, fainting, and heart failure. Diseases associated with SSS type 3: CHD, venous insufficiency, hypokalemia, atrial septal defect, scapula-peroneal myopathy, distal myopathy, "noncompact" LV. The only effective treatment for symptomatic and irreversible SSS is ECS implantation [12].

Type 4 (autosomal dominant SSS type 4). SSS type 4 is caused by a heterozygous mutation in the GNB2 gene (G-protein beta-2 subunit) in chromosome 7q22.1. The relationship between phenotype and gene is conditional. The inheritance pattern of SSS type 4 in the family is autosomal dominant [25]. SSS type 4 is characterized by early and progressive manifestation of SSS and AV conduction disorder. The following phenotypes associated with SSS type 4 are very rare (1 % of cases): sinus bradycardia, sinoatrial block, chronotropic failure, AVB, paroxysmal AF, syncope. AVB varies from mild to severe. Many require ECS implantation, but no cases of SCD have been reported [25].

## Perspectives of SSS gene therapy

ECS implantation is the most effective method for the treatment of symptomatic forms of SSS [13]. Currently, patients with SSS account for the vast majority of cases of ACS implantation [13]. This method improves the quality of life and increases its duration, which is determined by the nature and severity of concomitant organic heart disease, mainly myocardial dysfunction [12]. Currently there are no therapies for the treatment of the primary genetic cause in patients diagnosed with primary or chronic SSS [2, 5]. Despite the successes achieved in the development of implantable ECSs, it is still not possible to solve the problem of a biological pacemaker prototype completely, and the implantable ECSs produced to date are physiological only to a certain degree [12].

In the post-genomic era, an interest in the alternative molecular therapies for patients with SSS has emerged. In recent years, several groups of researchers have explored the possibility of creating a biological pacemaker that would eventually allow the replacement of implantable ECS [40]. Proposed strategies include gene therapy, transplantation of donor excitable myocardium, and delivery of modified embryonic stem cells to the heart.

Fetal and neonatal cardiac cells have been shown to functionally integrate and act as ectopic pacing cells when transplanted into the myocardium of dogs and pigs [41]. Gene therapy approaches include overexpression of beta-adrenergic receptors, inhibition of intrinsic potassium current rectification (IK1), insertion of HCN2 pacing gene into atrium using adenoviral or naked plasma vectors [5]. Another approach to the biological treatment of SSS is the engraftment of embryonic stem cells, which have differentiated, into atrial tissue [34].

A promising direction in the treatment of SSS is considered to be the stimulation of native pacemaker function or the creation of an ectopic pacemaker focus by gene transfer into existing cardiomyocytes or transplantation of pacemaker (including genetically modified) cells into the heart [41]. However, a complete solution to the challenges of creating a biological pacemaker should include a set of interventions to create a complex cellular and molecular structure similar to that of a biological SN.

Calcium-activated potassium channel 4 (HCN4) is known to be involved in cardiomyocyte automatism [28]. TRAM-34 (a selective blocker of HCN4 channels) has been shown to successfully reduce late postdepolarization and inhibit calcium turnover in induced human cardiomyocytes derived from pluripotent stem cells (hiPSC–CM) of patients with CPVT type 2 [42]. It has been shown that injection of TRAM-34 into CASQ2-D307H knockout mice results in a reduction of arrhythmias at rest and during exercise. Thus, TRAM-34, as a selective HCN4 channel blocker, has great therapeutic potential for humans with CASQ2 variants with loss-of-function [42].

Dysfunctional If-current causes an unusual exchange of calcium ions, disrupting the activity of pacemaker cells. If-current-deficient in mice have shown to have defects in pulse generation and conduction, which can be eliminated by a genetic deletion of cardiac muscarinic channels inactivated by G-protein (GIRK4) [23]. Although HCN4 and GIRK4 loss-of-function variants have been seen in SSS, the combination of silencing of both of these genes causes the phenotype of severe SSS associated with an AV blockade and ventricular arrhythmias [31].

The ability of the pharmacological inhibition of the IKACh channel to improve SN function has been demonstrated in a patient with a history of SSS [31]. Suppression of GIRK4 expression in human atrial myocytes effectively reduced IKACh density and, therefore, is a potential tool for the treatment of SSS. Thus, a gene therapy or pharmacological strategy targeting GIRK4 channels could be an important focus for SSS treatment in clinical practice.

HCN4 lentiviral gene transfer has demonstrated the bioengineering potential to allow the use of pacemaker cells. HCN4 transduction restored autonomic rhythm and increased sensitivity to autonomic regu-

lation in HCN4 transduced myocytes [5]. In addition, Myocyte enhancer factor-2 and activator protein-1 with binding sequences located on conserved non-coding sequence 13 (CNS13) are involved in HCN4 amplification through the HCN4 promoter [30] and can be used to activate HCN4 and thereby stimulate pacing activity of SN.

Thus, the problem of creating a biological pacemaker for SSS treatment is partly related to the complexity of genetic abnormalities and partly to the pleiotropy of genes. Many gene variants associated with SSS can exhibit several unrelated phenotypic traits. Currently, the clinical management of patients with SSS is limited to the relief of arrhythmia symptoms. Understanding the complexity of the genetics that contribute to the progression of SSS is for the development of new therapeutic strategies for this complex, life-threatening disorder.

## Conclusion

The familial form of SSS is a fairly common disease characterized by a wide range of cardiac manifestations from asymptomatic cases to the development of life-threatening arrhythmias. Given the polygenic origin of SSS, timely genetic testing is necessary, including family members of the proband. In this regard, identification of various gene mutations involved in the genesis of SSS is difficult, especially in when genetic overlap syndrome is present. In recent years, due to advances in genetic research on inherited cardiac arrhythmia syndromes, progress has been made in identifying gene therapy targets and in the development of genotype-specific therapy strategies for SSS. This creates prerequisites for the introduction into clinical practice of effective, safe methods of gene-modifying therap. The development of genotype-targeted pharmacotherapy, as well as functional capabilities of implantable pacemakers improvement as a prototype of biological pacemaker are also of high priority.

**Conflict of Interest.** None declared.

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