**Nephrology
Dialysis
Transplantation**

Teaching Point
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A young renal transplant recipient with unexplained resting sinus tachycardia, hypertension and β-blocker resistance

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Case

We report on a 32-year-old patient with reflux nephropathy diagnosed at the age of 11 years. Over the following 18 years, he developed end-stage renal disease (ESRD) and was started on haemodialysis in 2001. He had to be treated for severe hypertension with various medications including diuretics, β-blockers, α-blockers, calcium antagonists and angiotensin receptor blockers in various combinations. After 1 year of haemodialysis, the patient received a cadaveric renal transplant that immediately functioned well. Immunosuppression was installed according to the local standard protocol with basiliximab, cyclosporin A, mycophenolate mofetil and corticosteroids. The patient suffered from one episode of biopsy-proven acute tubulointerstitial rejection in the first week after transplantation, which was treated with three pulses of intravenous methylprednisolone. The subsequent course was uneventful, and corticosteroids could be stopped after 9 months.

The patient's family history revealed hypertension in his father and mother. The father suffered from a myocardial infarction at the age of 60, when hypertension was detected and treated. The mother has acknowledged mild to moderate hypertension for >10 years, but she was never treated and no further cardiovascular evaluation was performed.

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Pulse and blood pressure profiles

Already during haemodialysis this young and well-trained male patient suffered from moderate to severe hypertension. This problem persisted after transplantation and was always associated with high normal to high resting heart rate (80–115/min). Therefore, post-transplant antihypertensive treatment was based primarily on β-blockers. The course of resting blood pressure and heart rate under escalating doses of atenolol is shown in Figure 1. Since atenolol had no effect on both parameters even at 200 mg/day, it subsequently was replaced by carvedilol. This drug's combined α- and β-blocking effect led to an immediate amelioration of blood pressure. However, although the dose was maximally escalated, the patient's heart rate remained ~100/min (Figure 1).

Therefore, secondary causes of tachycardia were sought. Haemoglobin, thyrotropic hormone, fasting cortisol and glucose were within normal ranges. Cardiological examination revealed sinus tachycardia on resting electrocardiogram (ECG) and left ventricular hypertrophy in echocardiography, but no other abnormalities.

Based on pathophysiological considerations, the combination of tachycardia, hypertension and a clinical resistance to high dose β-blocker therapy pointed to a gain of function of the β1 adrenergic receptor. This hypothesis was evaluated in two respects: (i) a genetic analysis of the two most frequent polymorphisms of the β1 adrenergic receptor was performed (see below); and (ii) clinically: diltiazem, a class II Ca channel blocker with inhibitory effects on atrioventricular conduction independent of β1 adrenergic receptors, was added to the otherwise unchanged drug therapy (Figure 1). This efficiently lowered the patient's resting heart rate as measured by 24 h ECG analysis before and after introduction of diltiazem (mean pulse drop of 20/min).
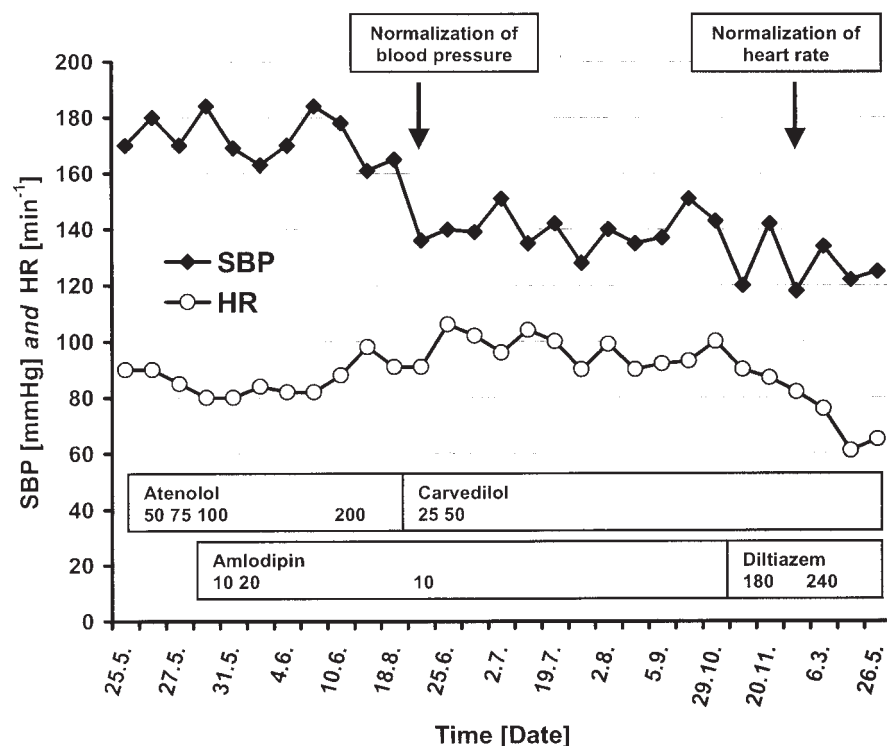
Fig. 1. Heart rate (HR) and systolic blood pressure (SBP) course. Heart rate (circles) and systolic blood pressure (diamonds) are indicated for the first year after renal transplantation. Medications with an effect on blood pressure and/or heart rate (name and total daily dose) are mentioned in separate boxes: atenolol is a β1-selective β-blocker, carvedilol a combined β- and α-blocker, amlodipin a class I calcium channel blocker and diltiazem a class II calcium channel blocker with inhibitory effects on atrioventricular conduction.

Molecular analysis of two β1 adrenergic receptor polymorphisms

In 1999, two important β1 adrenergic receptor polymorphisms were identified: one at amino acid position 49 (Ser49Gly) and one at position 389 (Arg389Gly) [1]. Whereas homozygosity for the serine allele at position 49 is associated with higher resting heart rate [2], homozygosity for arginine at position 389 is linked to elevated blood pressure and elevated heart rate [3].

Therefore, we analysed these two polymorphisms in our patient and his parents according to the original description in 1999 [1] with modified primers. Briefly, genomic DNA was extracted from EDTA blood. The polymorphic loci were amplified by polymerase chain reaction (PCR) with the following primers: for the Ser49Gly locus, upstream 5′-GTCGCCGCCCGCCT CGTT-3′ and downstream 5′-CCATGCCCGCTGTC CACTGCT-3′; and for the Arg389Gly locus, upstream 5′-GGCCTTCAACCCCATCATCTA-3′ and downstream 5′-CCGGTCTCCGTGGGTCGCGT-3′ [2]. The conditions for the PCRs were as follows: initial denaturation at 94°C for 5 min, 40 cycles with 1 min at 94°C, 1 min at 68°C (Ser49Gly) or 63°C (Arg389Gly) and 2 min at 72°C with final extension for 10 min.

The PCR products were then directly sequenced, and the results are shown in Figure 2. The figure shows electropherograms from an automated sequence analyser, where each of the four bases (A, T, C and G) is represented by an individual colour. In Figure 2A,

nucleic acid position 145 is shaded. The electrophero-gram shows a single green peak for A in all three individuals, meaning that they are all homozygous for A at this position. The triplet AGC codes for the amino acid serine at position 49 (Ser49Gly locus). In Figure 2B, the nucleic acid position 1165 is shaded. In our index patient and his mother, we observed a single peak for C, meaning that they are homozygous for C at this position. However, the father shows a double peak for C (blue, lower peak) and G (black, higher peak). This means that he is heterozygous at this position. The triplet CGA codes for the amino acid arginine at position 389 (Arg389Gly locus), whereas for the father the triplet GGA codes for glycine on one and CGA for arginine on the other allele.

Thus, our patient and his mother were both homo-zygous for serine at position 49 and for arginine at position 389, therefore harbouring the highest risk constellation for tachycardia and hypertension at these two loci. In contrast, the patient's father was heterozygous for arginine at position 389, but also homozygous for serine at 49.

Discussion

Cardiovascular diseases are the most frequent cause of hospitalizations and death in industrialized countries. Several modifiable risk factors such as hypertension,
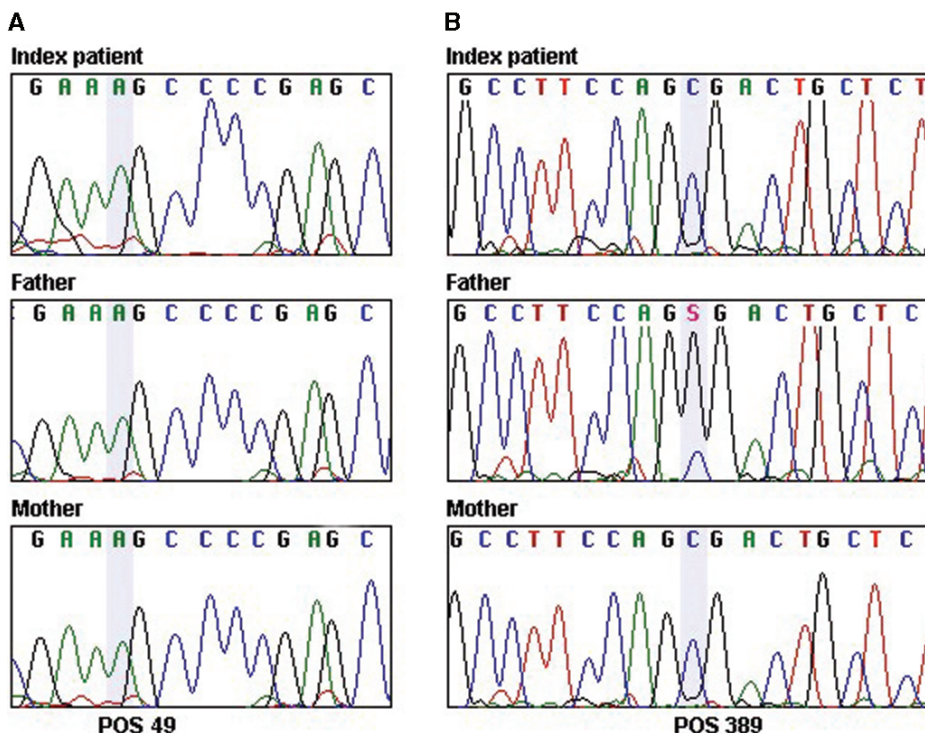
Fig. 2. Sequencing results of β1 receptor polymorphism analysis for the index patient and his father and mother. Genomic DNA of the two main polymorphic loci of the human β1 receptor (Ser49Gly and Arg389Gly) was amplified by PCR. Products were sequenced according to standard procedures (see text). (**A**) Ser49Gly polymorphism: the index patient, his father and his mother were homozygous for serine at position 49. The important nucleic acid position 145 is shaded. A single peak for A is seen in all three individuals. AGC at this position codes for serine at position 49. (**B**) Arg389Gly polymorphism: the index patient and his mother were homozygous for arginine at position 389, whereas the father was heterozygous for arginine and glycine at this position. The important nucleic acid position 1165 is shaded. A single peak for C is seen in the index patient and his mother, whereas a double peak for C and G is observed in the father (S stands for C or G in the sequence analysis, which results in arginine or glycine for the encoded amino acid).

hyperlipidaemia, diabetes mellitus and smoking have been identified. Familial history of coronary heart disease or stroke is another important risk factor, which until now could not be modified. In the era of molecular medicine, several genetic causes of cardio-vascular diseases have been recognized. Some of them influence the mentioned cardiovascular risk factors, e.g. mutations of the low-density lipoprotein receptor, genetic variants of diabetes mellitus (maturity onset diabetes of the young type 1–6) and angiotensin-converting enzyme (ACE) polymorphisms predisposing to hypertension [4]. Others lead directly to structural (e.g. genetic forms of hypertrophic and dilated cardiomyopathy with myosin or troponin mutations) or arrhythmogenic heart disease (e.g. familial forms of long-QT syndrome with ion channel mutations) [5]. In recent years, reports on adrenergic receptor polymorphisms (α1, β1, β2, β3) and their influence on blood pressure, heart rate and prognosis of cardiovascular disease have been published. The human β1 adrenergic receptor was cloned in 1987 [6], but the significance of polymorphisms in this gene for the course of cardiovascular disease was recognized only 10 years later. The two main polymorphisms of the β1 adrenergic receptor (Ser49Gly and Arg389Gly) are associated with elevated heart rate and/or hypertension [2,3].

We describe a young patient with unexplained resting tachycardia and hypertension, in which pathophysiological considerations together with specific pharmacological treatment interventions have led to genetic analysis and detection of homozygosity for two β1 adrenergic receptor polymorphisms. These polymorphisms are associated with a higher risk for hypertension and tachycardia [2,3] and may explain the patient's resistance to β-blocker therapy. The clinical findings exactly fit *in vitro* studies on the cellular level, where a gain of function could be demonstrated for the arginine *vs* glycine allele at position 389 (enhanced agonist-induced adenylyl cyclase activity [7]) and also for the serine *vs* glycine allele at position 49 (reduced agonist-promoted receptor downregulation [8]).

Among a long list of antihypertensive drug classes, β-blockers and thiazide diuretics represent the standard treatment for patients with simple primary hypertension [9]. Concomitant diseases and side effects are the major determinants for the choice of other drug classes. However, with the detection of ACE polymorphisms, which are frequent among African Americans and lead to a partial resistance to ACE inhibitor therapy [4], the importance of genetic factors for the individual response to cardiovascular drug treatments has become evident. In our patient, we found a comparable situation with resistance to β-blocker therapy due to

β1 adrenergic receptor polymorphisms. The clinical importance of adrenergic receptor polymorphisms has only been recognized very recently by clinical studies that demonstrated their association with the incidence [10] and mortality [11] of congestive heart failure, and their predictive value for exercise capacity in patients with heart failure [12].

In conclusion, gain of function of the β1 adrenergic receptor seems to be detrimental for the heart muscle, which was experimentally proven by the development of heart failure in β1 adrenergic receptor transgenic mice [13]. In contrast, its inhibition by β-blocker therapy has highly beneficial effects in patients with heart failure [14]. Given the large number of patients with arterial hypertension and unexplained systolic heart failure, screening for adrenergic receptor polymorphism might have increasing clinical importance in the near future.

Teaching point

Adrenergic receptor polymorphisms are an increasingly recognized cause of unexplained tachycardia, hypertension and congestive heart failure. These polymorphisms add to a growing list of genetic factors that determine an individual's response to antihypertensive pharmacotherapy. It will be a major task in the future to integrate this information in order to maximally tailor drug therapy to each individual's genetic profile. An optimal effect–side effect relationship should then result, and our patient may serve as an early example for this attempt.

Conflict of interest statement. None declared.

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