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Genetic variability of the extraneuronal monoamine transporter EMT (SLC22A3)

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Abstract The extraneuronal monoamine transporter EMT (HGNC Nomenclature SLC22A3) is the molecular correlate of the classical uptake₂ system responsible for the non-neuronal inactivation of circulating and centrally released catecholamines. Because of its functional profile and expression pattern, EMT is regarded as a candidate gene for diseases related to the sympathetic nervous system and neuropsychiatric disorders. We describe the first investigation of the genetic variability of the EMT gene in human. Six single-nucleotide substitutions and one deletion were detected within the assumed core promoter, the exonic and flanking intronic sequences and the 3'-untranslated region in 100 Caucasian individuals. No amino acid changes were found and Tajima's D was positive ($D = 2.91$; $P < 0.01$). However, the synonymous nucleotide substitution 1233G → A might serve as a cryptic splice acceptor site. Analysis of linkage disequilibrium between polymorphisms yielded 12 possible haplotypes accounting for more than 90% of all haplotypes. Knowledge of the sequence variation and frequency of the underlying polymorphisms in this member of the amphiphilic solute facilitator family of transporters provides the basis for subsequent association studies and candidate gene approaches.

Keywords SNP · SLC22A3 · EMT · Caucasians · Frequency of polymorphisms

Introduction

Catecholamine signal transduction regulates various physiological functions, such as blood pressure, lipid metabolism, pituitary-adrenocortical axis, immune system and behaviour (Mark 1996; Arner 2001; Bugajski et al. 1998; Sanders and Straub 2002; Brunner et al. 1993). Disorders in catecholaminergic signalling are related to common diseases, such as hypertension, congestive heart failure, Parkinson's disease and addiction (Rumantir et al. 2000; Packer et al. 1996; Wooten and Trugman 1989; Mash et al. 2002). The effect of released catecholamines is terminated by reuptake mechanisms via two distinct transport systems. In addition to high-affinity low-capacity transport into releasing neurons (Pacholczyk et al. 1991), the inactivation of catecholamines is mediated by a low-affinity high-capacity non-neuronal system, originally named uptake₂ (Iversen 1965).

The extraneuronal monoamine transporter EMT has been identified as the molecular correlate that participates in the inactivation of circulating and centrally released catecholamines (Graefe et al. 1998; Russ et al. 1996; Schömig et al. 1998). Furthermore, as a member of the amphiphilic solute facilitator (ASF) family EMT is thought to play a pivotal role in the elimination of cationic xenobiotics (Chen et al. 1999).

Because of its functional profile and expression in sympathetically innervated tissues, EMT is a candidate gene for common disorders such as hypertension, obesity or neuropsychiatric diseases (Gründemann et al. 1998; Eisenhofer 2001). The pathogenetic significance of polymorphisms has been reported for neuronal catecholamine transporters (Shannon et al. 2000; Lesch et al. 1996).

The path for studying EMT has recently been paved by revealing its genetic structure (Gründemann and Schömig 2000). We report the first investigation of the genetic variability of EMT in 100 unrelated individuals of Caucasian origin.

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Materials and methods

Subjects

Genomic DNA from 100 unrelated healthy Caucasian individuals was used to sequence directly the assumed core promoter region 500 bp upstream of the transcription starting point and all 11 exons including exon-intron boundaries and part of the 3'-untranslated region (3'-UTR). The sample size permitted the detection of alleles at a frequency of 0.05 with a power of more than 99.9% and the detection of alleles at a frequency of 0.01 with a power of more than 86%. Genomic DNA was extracted by the Blood and Cell Midi Kit (QIAGEN).

Informed consent

All persons gave their written informed consent prior to inclusion in the study. The study design was approved by the appropriate ethics committee.

Basic molecular biology

Polymerase chain reaction (PCR) was performed for promoter and exon 1 (5 min at 94°C as the denaturation step, 40 cycles of 1 min at 94°C and 2 min at 68°C, and 10 min at 72°C as the final elongation) in 50- μ l reaction mixtures containing 100 ng genomic DNA, 1 $\times$ Pfx amplification buffer, 1 mM MgSO₄, 0.5 μ M each primer, 0.3 mM each dNTP, 1 M formamide, 5% dimethylsulphoxide and 1.25 U Platinum Pfx DNA Polymerase (Life Technologies). PCR for exons 2–11 (34 cycles of 30 s at 94°C, 1 min at 60°C, and 1 min at 72°C, and 10 min at 72°C as the final elongation) was carried out in 50- μ l reaction mixtures containing 100 ng genomic DNA, 10 mM TRIS-HCl (pH 9.0), 50 mM KCl, 2.3 mM MgCl₂, 0.5 μ M each primer, 0.2 mM each dNTP and 1.5 U Taq DNA polymerase (Promega, Storage Buffer A). Before being used in PCR, genomic DNA was restricted by EcoRV. PCR products were separated on UV-protected 1.6% agarose gels (Gründemann and Schömig 1996), visualized with ethidium bromide under UV-light and purified by employing the QiaQuick Gel Extraction kit (QIAGEN). Oligonucleotides used for amplification are shown in Table 1.

Direct sequencing was carried out with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction v2.0 (Applied Biosystems) under the following conditions. Promoter and exon 1 (5 min at 98°C for preincubation without Reaction Mix, short incubation on ice, addition of Reaction Mix, 5 min at 94°C as an initial denaturation step, 25 cycles of 1 min at 94°C and 4 min at 68°C with a ramping speed of 1°C/s, and 10 min at 68°C as the final elongation) were sequenced in 20- μ l reaction mixtures containing 50 ng PCR product as template, 4 μ l Terminator Ready Reaction Mix, 0.3 μ M sequencing primer and 5% DMSO. Exons 2–11 (25 cycles of 10 s at 96°C, 30 s at 55°C and 4 min at 60°C with a ramping speed of 1°C/s) were sequenced in 10- μ l reaction mixtures

containing 50 ng PCR product as template, 2 μ l Terminator Ready Reaction Mix and 0.3 μ M sequencing primer. The sequencing products were purified by employing Sephadex G50 superfine (Sigma-Aldrich) and MAHVN030 96-well plates (Millipore). Sequencing analysis was performed on an Applied Biosystems Genetic Analyzer 3100 capillary sequencer. Pairwise sequence alignments were performed with the MT Navigator PPC and Edit View 1.0.1 programs (ABI).

For direct sequencing the following oligonucleotides were used (5'-end first): CTC GTC GAA GGA GGG CAT GGT, ACG CGC AAG GGC, CGG GCG GTG CGG CCT GAC TAC G (promoter), CCG CCG GCT GGG TCC GCG, CCT CCC CTG GCG GCC (exon 1), CCT CCC CTG GCG GCC AGC GTC TCA CA (exon 2), GAG GAA GGT TGA TTA ACT AGC ATT T (exon 3), GTA ACA GGT GTA ACA TCT CTG TAT T (exon 4), GCA ACA TTA AGT ACA CAA GAA GCA ACA AAA (exon 5), TAG GTG TTT TCA AGT CCT TT (exons 6/7), CTG AGC TAA GGA AAC GAC TTC ATA GTT TT (exon 8), CAA AGT ATC TTC ACA CTT CCT TTG GTT TT (exon 9), GTC ATT CTT TCC CCC GTG GTT TT (exon 10), CCT TAG AGT TGG GTT TTC ATG AAT GT (exon 11).

Genbank accession numbers

Reference sequences NT_029991 and XM_011436 are available from the GenBank database.

Statistical analysis

Haplotype frequency was estimated by using the EH plus program (Xie and Ott 1993; Terwilliger and Ott 1994). For the calculation of the population mutation parameter θ , based on the number of segregating sites, nucleotide diversity π , Tajima's D, Lewontin's coefficient D' (Lewontin 1984) and standardized linkage disequilibrium coefficient r (Hill and Robertson 1968), DnaSP version 3.53 software was employed (Rozas and Rozas 1999). Pairwise linkage disequilibrium was calculated as $D = x_{ij} - p_i p_j$, where x_{ij} is the frequency of haplotype A₁ B₁, and p_1 and p_2 are the frequencies of alleles A₁ and B₁ at loci A and B, respectively (Lewontin and Kojima 1960). Lewontin's coefficient D' is given by D/D_{max} , where $D_{max} = \min[p_1 p_2, q_1 q_2]$ when $D < 0$ and $D_{max} = \min[q_1 p_2, p_1 q_2]$ when $D > 0$ (Lewontin 1984). The standardized linkage disequilibrium coefficient, r, is given by $D/(p_1 p_2 q_1 q_2)^{1/2}$, where q_1 and q_2 are the frequencies of the other alleles at loci A and B, respectively (Hill and Robertson 1968).

Results

Genetic variation of the EMT gene

Six single-nucleotide substitutions and one deletion were identified within the putative core promoter, all 11

Table 1 Oligonucleotides used for amplification of the EMT gene

Fragment	Forward (5' → 3')	Reverse (5' → 3')	Size (bp)
Promoter	GCGCAGGAGGCCTCCGCACGCGCAA	GCGAAGGTGACGCCCGTCAGGCACA	673
Exon 1	AGGCCGCCGGCTGGGTCCGCGGGTC	CGACCTCCCCTGGCGGCCAGCGTCTCACA	595
Exon 2	TTACATCCTTTG CAGTTATTTAAACAAA	CCAACCAAGTGTGGATGTCATGAAT	453
Exon 3	GAGCTCTTTTCTGTCTTTCTCAAAAAG	CTTCTCTCCTTCTCCCACTCACAGAAA	987
Exon 4	GTATCAAGAAGGACACCTCCATC	GTCTGTTTGGAGTCTAATTTCTGC	904
Exon 5	GGGACCTATTATCAACTCTTATTGACTCCTAAA	GGTAGTGTGTGTGTGTGTGTATACATA	533
Exon 6/7	TAATGACAAAGTCTATACTATGCAAGTTT	CCTATACTTGATTGTGGATTTAGGTGTT	576
Exon 8/9	GGAGGCCACTAAGCAAATACTTTTT	CTGAATTGGCTCTCAAAACTGAAATAGT	1257
Exon 10	GGTAAGGGAGAGCTGGGGCATTGAT	ATTGAGAAAATTGAATATCCACAGGG	350
Exon 11	CCCCTGTCTAAGGTCTGAACACAGACAAAA	CCCTTGACCAGATTTGATGAACACATTT	667

Table 2 Polymorphisms of the EMT gene in 100 Caucasian individuals

Polymorphism ^a	Type of change	Genotype			Allele frequency		dbSNP number	Protein domain
-29A → G	Noncoding	AA	AG	GG	A	G	rs555754	–
360T → C	Synonymous (Arg120Arg)	TT	TC	CC	T	C	rs668871	Extracellular
603T → C	Synonymous (Phe201Phe)	TT	TC	CC	T	C	ss5112516	TMD 3
1233G → A	Synonymous (Ala411Ala)	GG	GA	AA	G	A	rs2292334	Cytoplasmic
IVS9 –60delC	Noncoding	CC	C –	– –	C	–	ss5112512	–
IVS9 –50A → G	Noncoding	AA	AG	GG	A	G	rs2457574	–
1734C → T	Noncoding	CC	CT	TT	C	T	rs1810126	–
		37	54	9	0.640	0.360		

^aThe nomenclature of mutations follows the recommendations of den Dunnen and Antonarakis (2001)

exons, exon-intron boundaries and part of the 3'-UTR in 100 healthy unrelated individuals of Caucasian descent. Interestingly, no non-synonymous nucleotide substitutions and solely transitions were found. Polymorphisms 603T → C and IVS9 –60delC were novel and had not previously been published by the National Center for Biotechnology Information. The deletion is situated within a short 8-bp palindromic sequence that might serve as a hot spot to promote mutations (Table 2).

The nucleotide diversity π was calculated $(6.7 \pm 4.0) \times 10^{-4}$ for 3686 bp genomic sequence and $(7.1 \pm 4.1) \times 10^{-4}$ for 1926 bp exonic sequence, i.e. there was one single-nucleotide polymorphism (SNP) every 1550 bp on average. The mutation parameter θ was estimated as $(2.8 \pm 1.3) \times 10^{-4}$ for the whole sequence and $(3.5 \pm 1.9) \times 10^{-4}$ for the exonic region. At synonymous sites, π and θ were computed as $(23.3 \pm 13.4) \times 10^{-4}$ and $(11.9 \pm 4.8) \times 10^{-4}$, respectively, whereas at non-synonymous sites π and θ valued 0, since there were no amino acid changes.

Remarkably, Tajima's D for the genomic sequence investigated was 2.91, formally discarding the null hypothesis of selective neutrality at $P < 0.01$ (Tajima 1989).

No evidence for the functional importance of any of the polymorphisms detected could be found since there was only inheritance in Mendelian ratios. On the other hand, the G → A substitution at position 1233 in exon 7 changes the two codons for alanine GCGGCA to the sequence GCAGCA and opens the theoretical option for a cryptic splice acceptor site with the consequence of a loss of coding sequence and a frameshift further downstream (Fig. 1). The variant sequence CAG/CA matches the C₆₅A₁₀₀G₁₀₀/N rule for splice acceptor sites and is directly preceded by 10 pyrimidine bases. Additionally, further upstream there is a possible branch site (Strachan and Read 1999).

Haplotype analysis

Haplotypes may provide an even more powerful tool for detecting disease association (Stephens et al. 2001).

1233G

...TCTTACTAACCATTGAGCGCCTTGGACGACGCCTCCCTTTGC**G**Gc...

1233A

...TCTTACTAACCATTGAGCGCCTTGGACGACGCCTCCCTTTGC**A**G/C...

branch site 21 nucleotides 10 pyrimidines

Fig. 1 The 1233G → A polymorphism within exon 7 fulfils criteria of a cryptic 3'-splice acceptor site

Consequently, haplotype analysis utilizing an iterative procedure was performed yielding the occurrence of 20 possible haplotypes. Twelve of them (representing over 90% of calculated haplotypes) are displayed in Table 3.

Strong, but incomplete, linkage disequilibrium was detected among some of the six prevalent polymorphisms (Table 4). Recombination events, e.g. by cross-over or gene conversions, occurred in between segregating sites -29A → G/360T → C, 360T → C/1233G → A, 1233G → A/IVS9 –60delC, IVS9 –60delC/IVS9 –50G → A and IVS9 –50G → A/1734C → T.

Discussion

Catecholamine signalling pathways control elementary physiological functions, such as blood pressure homeostasis, affection and cognition. Catecholaminergic actions are cancelled by inactivation through neuronal and non-neuronal transport systems. The extraneuronal monoamine transporter EMT (the correlate of the classical uptake₂ system) has been hypothesized to contribute to the pathogenesis of common autonomic and psychiatric disorders.

We have carried out the first analysis of the genetic variation of EMT and detected six SNPs and one deletion within the presumed core promoter and coding and adjacent non-coding sequence in 100 Caucasians. The

Table 3 Estimation of haplotype frequency among 100 Caucasian individuals with the assumption of association between markers. Haplotypes at an estimated frequency of greater than 2.5% representing over 90% of sequences are shown

Haplotype	-29 A → G	360 T → C	603 T → C	1233 G → A	IVS9 -60delC	IVS9 -50A → G	1734 C → T	Haplotype frequency
*1	A	C ^a	T	G	C	G ^a	C	0.177
*2	G ^a	C ^a	T	A ^a	delC ^a	G ^a	T ^a	0.138
*3	G ^a	T	T	A ^a	delC ^a	A	T ^a	0.123
*4	A	T	T	G	C	A	C	0.099
*5	G ^a	C ^a	T	G	C	A	C	0.089
*6	A	T	T	G	delC ^a	A	T ^a	0.067
*7	G ^a	T	T	G	C	G ^a	C	0.056
*8	G ^a	T	T	A ^a	delC ^a	G ^a	T ^a	0.042
*9	A	T	T	G	C	G ^a	C	0.039
*10	A	C ^a	T	G	C	A	C	0.028
*11	A	T	T	A ^a	delC ^a	A	T ^a	0.026
*12	A	C ^a	T	A ^a	delC ^a	A	T ^a	0.025

^aMinor alleles

Table 4 Strength of linkage disequilibrium (D' Lewontin's coefficient, r standardized linkage disequilibrium coefficient). Nucleotide substitution 603T → C was disregarded because of a too low allele frequency. No more than two significant digits were considered

Polymorphism		360T → C	1233G → A	IVS9 -60delC	IVS9 -50G → A	1734C → T
-29A → G	D'	-0.02	0.57	-0.41	0.01	0.34
	r^2	0	0.23	0.16	0	0.11
360T → C	D'		0.05	-0.19	0.36	0.18
	r^2		0	0.03	0.13	0.03
1233G → A	D'			-1.00	-0.07	0.98
	r^2			0.67	0	0.73
IVS9 -60delC	D'				-0.09	-1.00
	r^2				0	0.87
IVS9 -50G → A	D'					0.06
	r^2					0

1233G → A polymorphism might serve as a cryptic splice acceptor site within certain tissues; this requires further investigation. Nonetheless, as a striking result, there were no amino acid changes and Tajima's D was significantly positive. Interestingly, a recent analysis of the genetic variation of the nearby transporter genes OCT1 and OCT2 has revealed the occurrence of amino acid substitutions (Saito et al. 2002; Leabman et al. 2002). The absence of non-synonymous mutations in the EMT gene and the positive value of Tajima's D might result from selective mechanisms that operated during evolution of the human species against amino acid changes (Tajima 1989) but could also be caused by demographic factors such as population subdivision (Simonsen et al. 1995). The investigation of different ethnic groups might detect population-specific non-synonymous polymorphisms within the EMT gene that are not prevalent in Caucasians. Furthermore, Tajima's test makes various assumptions, e.g. no recombination or random mating, both of which are violated within human populations.

The nucleotide diversity π and mean SNP density were only slightly lower than that reported for chromosome 6 (7.44×10^{-4}) and whole-genome mean SNP spacing (1209 bases per SNP; International SNP Map Working Group 2001; http://www.ncbi.nlm.nih.gov/SNP/snp_summary.cgi).

On the other hand, the mean value of the mutation parameter θ was well below the estimates for θ (9.6×10^{-4} and 8.3×10^{-4}) given by others (Stephens et al. 2001; Halushka et al. 1999). This difference can in part be explained by the investigation of a large number of genes and ethnically diverse samples in these studies.

Lack of protein sequence variation may reflect the crucial role of EMT within the human organism. The investigation of patients with candidate disorders might reveal deleterious polymorphisms that occur at low prevalence and that were not found in our study of healthy individuals.

Nevertheless, the data of non-coding polymorphisms can successfully be utilized to perform linkage disequilibrium analysis and to extract information about disease association, as provided by the example of the calpain 10 gene and type 2 diabetes mellitus (Horikawa et al. 2000). The data at hand further expand our knowledge about this member of the ASF family of transporters and provide the basis for subsequent association studies and candidate gene approaches.

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References

- Arner P (2001) Genetic variance and lipolysis regulation: implications for obesity. *Ann Med* 33:542–546
- Brunner HG, Nelen M, Breakefield XO, Ropers HH, Oost BA van (1993) Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. *Science* 262:578–580
- Bugajski J, Borycz J, Gadek-Michalska A (1998) Involvement of the central noradrenergic system in cholinergic stimulation of the pituitary-adrenal response. *J Physiol Pharmacol* 49:285–292
- Chen ZP, Wang G, Huang Q, Sun ZF, Zhou LY, Wang AD, Panasci LC (1999) Enhanced antitumor activity of sarCNU in comparison to BCNU in an extraneuronal monoamine transporter positive human glioma xenograft model. *J Neurooncol* 44:7–14
- Dunnen JT den, Antonarakis SE (2001) Nomenclature for the description of human sequence variations. *Hum Genet* 109:121–124
- Eisenhofer G (2001) The role of neuronal and extraneuronal plasma membrane transporters in the inactivation of peripheral catecholamines. *Pharmacol Ther* 91:35–62
- Graefe KH, Friedgen B, Wolfel R, Schömig E (1998) Removal of circulating catecholamines by extraneuronal amine transport systems. *Adv Pharmacol* 42:360–363
- Gründemann D, Schömig E (1996) Protection of DNA during preparative agarose gel electrophoresis against damage induced by ultraviolet light. *Biotechniques* 21:898–903
- Gründemann D, Schömig E (2000) Gene structures of the human non-neuronal monoamine transporters EMT and OCT2. *Hum Genet* 106:627–635
- Gründemann D, Schechinger B, Rappold GA, Schömig E (1998) Molecular identification of the corticosterone-sensitive extraneuronal catecholamine transporter. *Nat Neurosci* 1:349–351
- Halushka MK, Fan JB, Bentley K, Hsie L, Shen N, Weder A, Cooper R, Lipshutz R, Chakravarti A (1999) Patterns of single-nucleotide polymorphisms in candidate genes for blood-pressure homeostasis. *Nat Genet* 22:239–247
- Hill WG, Robertson A (1968) Linkage disequilibrium in finite populations. *Theor Appl Genet* 38:226–231
- Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PE, del Bosque-Plata L, Oda Y, Yoshiuchi I, Colilla S, Polonsky KS, Wei S, Concannon P, Iwasaki N, Schulze J, Baier LJ, Bogardus C, Groop L, Boerwinkle E, Hanis CL, Bell GI (2000) Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 26:163–175
- International SNP Map Working Group (2001) A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* 409:928–933
- Iversen LL (1965) The uptake of catechol amines at high perfusion concentrations in the rat isolated heart: a novel catechol amine uptake process. *Br J Pharmacol* 25:18–33
- Leabman MK, Huang CC, Kawamoto M, Johns SJ, Stryke D, Ferrin TE, DeYoung J, Taylor T, Clark AG, Herskowitz I, Giacomini KM (2002) Polymorphisms in a human kidney xenobiotic transporter, OCT2, exhibit altered function. *Pharmacogenetics* 12:395–405
- Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 274:1527–1531
- Lewontin RC (1984) The interaction of selection and linkage. I. General considerations; heterotic models. *Genetics* 49:49–67
- Lewontin RC, Kojima K (1960) The evolutionary dynamics of complex polymorphisms. *Evolution* 14:450–472
- Mark AL (1996) The sympathetic nervous system in hypertension: a potential long-term regulator of arterial pressure. *J Hypertens Suppl* 14:S159–S165
- Mash DC, Pablo J, Ouyang Q, Hearn WL, Izenwasser S (2002) Dopamine transport function is elevated in cocaine users. *J Neurochem* 81:292–300
- Pacholczyk T, Blakely RD, Amara SG (1991) Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter. *Nature* 350:350–354
- Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilbert EM, Shusterman NH (1996) The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. US Carvedilol Heart Failure Study Group. *N Engl J Med* 334:1349–1355
- Rozas J, Rozas R (1999) DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics* 15:174–175
- Rumantir MS, Kaye DM, Jennings GL, Vaz M, Hastings JA, Esler MD (2000) Phenotypic evidence of faulty neuronal norepinephrine reuptake in essential hypertension. *Hypertension* 36:824–829
- Russ H, Staust K, Martel F, Gliese M, Schömig E (1996) The extraneuronal transporter for monoamine transmitters exists in cells derived from human central nervous system glia. *Eur J Neurosci* 8:1256–1264
- Saito S, Iida A, Sekine A, Ogawa C, Kawauchi S, Higuchi S, Nakamura Y (2002) Catalog of 238 variations among six human genes encoding solute carriers (hSLCs) in the Japanese population. *J Hum Genet* 47:576–584
- Sanders VM, Straub RH (2002) Norepinephrine, the beta-adrenergic receptor, and immunity. *Brain Behav Immun* 16:290–332
- Schömig E, Russ H, Staudt K, Martel F, Gliese M, Gründemann D (1998) The extraneuronal monoamine transporter exists in human central nervous system glia. *Adv Pharmacol* 42:356–359
- Shannon JR, Flattem NL, Jordan J, Jacob G, Black BK, Biaggioni I, Blakely RD, Robertson D (2000) Orthostatic intolerance and tachycardia associated with norepinephrine-transporter deficiency. *N Engl J Med* 342:541–549
- Simonsen KL, Churchill GA, Aquadro CF (1995) Properties of statistical tests of neutrality for DNA polymorphism data. *Genetics* 141:413–429
- Stephens JC, Schneider JA, Tanguay DA, Choi J, Acharya T, Stanley SE, Jiang R, Messer CJ, Chew A, Han JH, Duan J, Carr JL, Lee MS, Koshy B, Kumar AM, Zhang G, Newell WR, Windemuth A, Xu C, Kalbfleisch TS, Shaner SL, Arnold K, Schulz V, Drysdale CM, Nandabalan K, Judson RS, Ruano G, Vovis GF (2001) Haplotype variation and linkage disequilibrium in 313 human genes. *Science* 293:489–493
- Strachan T, Read AP (1999) Human molecular genetics, 2nd edn. BIOS, Oxford
- Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123:585–595
- Terwilliger J, Ott J (1994) Handbook of human genetic linkage. Johns Hopkins University Press, Baltimore
- Wooten GF, Trugman JM (1989) The dopamine motor system. *Mov Disord* 4:S38–S47
- Xie X, Ott J (1993) Testing linkage disequilibrium between a disease gene and marker loci (abstract). *Am J Hum Genet* 53:1107