PURINERGIC SIGNALING IN ATHEROSCLEROSIS AND RESTENOSIS

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Background

The concept of purinergic signaling, i.e. ATP acting as an extracellular signaling molecule, was put forward in 1972, when evidence was presented in support of ATP as a neurotransmitter in non-adrenergic, non-cholinergic (NANC) nerves in the gut, bladder, and some blood vessels [1]. After considerable early resistance purinergic signaling is now fully accepted for both neuronal and non-neuronal cells [2-5].

Extracellular receptors for purines and pyrimidines were recognized and currently four P1 (adenosine) subtypes, seven P2X ion channel and eight P2Y G protein-coupled receptor subtypes have been cloned and characterized [6-9]. Both short-term purinergic signaling in neurotransmission, neuromodulation, exocrine and endocrine secretion, platelet aggregation, mechanosensory transduction, and long-term (trophic) purinergic signaling of cell proliferation, differentiation, migration, and death during development and regeneration has been described [10,11]. There is increasing evidence for roles for purinergic signaling in pathological conditions [3].

In the vascular system, purinergic signaling is involved in dual control of vascular tone and remodeling by ATP released as a cotransmitter from perivascular nerves and released from endothelial cells during changes in blood flow (shear stress) and hypoxia to act on both P2X and P2Y receptors on endothelial cells to release nitric oxide resulting in vasodilatation [12-18]. Uridine adenosine tetraphosphate (Up_4A) is also released from endothelial cells in kidney and lung to cause endothelium-dependent vasoconstriction, perhaps largely via $P2X_1$ receptors [19,20].

Atherosclerosis and Restenosis

There is growing evidence that ATP signaling is involved in atherosclerosis [14,21-24]. Adenosine and ATP have a number of cardiovascular protective effects in addition to vasodilatation, including the promotion of endothelial and smooth muscle cell proliferation (see Figure 1) and an increase in the expression of vascular endothelial growth factor (VEGF) mRNA, which plays an important role in the development of intimal thickening during arterial diseases, such as atherosclerosis, and in restenosis after angioplasty, and in the growth of new vessels that takes place during wound healing and in tumors [14]. Hypoxia is an important stimulus to vascular growth and it is believed that ATP, which is released from endothelial cells during hypoxia and its breakdown product adenosine, has important roles as mediators of blood vessel growth.

An early study reported that adenosine produces changes in cyclic AMP and DNA synthesis in cultured arterial smooth muscle cells and suggested that this might result in the regulation of cell proliferation [25]. The authors speculated that adenosine could be one of several regulatory factors in the development of atherosclerosis and might also regulate the

release of a smooth muscle mitogen, platelet-derived growth factor. There is now supporting evidence that adenosine does indeed regulate smooth muscle cell proliferation in angiogenesis [26]. Endothelial cell proliferation is mediated by A_{2A} and A_{2B} receptors and some of the mitogenic effects are mediated via the modulation of VEGF signaling. There is evidence that A_{2B} receptors mediate inhibition of the growth of human aortic smooth muscle cells [27].

ATP and ADP also mediate cell proliferation via P2 receptors. ATP and ADP stimulate DNA synthesis and cell proliferation of porcine aortic smooth muscle cells via activation of P2Y receptors [28]. UTP also has powerful mitogenic actions on vascular smooth muscle, and since the mitogenic effects of UTP and ATP were approximately equipotent [29,30] this would suggest that the receptor involved is either a $P2Y_2$ or $P2Y_4$ subtype. There was upregulation of vascular smooth muscle $P2Y_2$ receptors by mitogen-activated protein kinase (MAPK)-dependent growth factor, which the authors suggested may be important in atherosclerosis and neointimal formation after balloon angioplasty [12]. Upregulation and activation of $P2Y_2$ receptor has been shown to mediate intimal hyperplasia in rabbit carotid artery [31], basilar artery [32], and diabetic pig coronary artery [33]. It has been proposed that upregulation of $P2Y_2$ receptors may be a useful diagnostic indicator for the early stages of atherosclerosis [34]. ATP and ADP have also been shown to stimulate endothelial cell migration and proliferation [35,36], probably via P2Y receptors.

Vascular injury represents a critical initiating event in the pathogenesis of various vascular diseases. Large amounts of ATP are released from injured cells and, as described above, ATP and adenosine have potent actions on smooth muscle and endothelial cell growth, migration, proliferation, and death. Atherosclerotic damage results in the disappearance of endothelium-dependent responses to ATP [3]. The release of ATP from endothelial cells has also been claimed to be impaired in atherosclerotic arteries and long-term supplementation with a high cholesterol diet decreases the release of ATP. Clinical trials with clopidogrel and ticlopidine ($P2Y_{12}$ receptor antagonists) in patients with atherosclerotic disease have shown significant benefit compared with aspirin.

Natural killer cells represent the main source of interferon-γ that contributes to atherosclerotic plaque progression and instability. ATP release from endothelial cells by shear stress and enhanced in inflammatory states [37] might represent protective mechanisms for killer cell-mediated plaque formation [38].

Apoptotic cell death is recognized to occur in a number of vascular diseases, including atherosclerosis, restenosis, and hypertension [39,40]. ATP releases histamine from mast cells and releases inflammatory cytokines such as interleukin-1 from immune cells via $P2X_7$ receptors. In addition, occupation of P2Y receptors leads to prostaglandin and cyclo-oxygenase-2 (COX-2) synthesis [41], both involved in inflammatory processes. ATP-induced COX-2 expression is via p42/p44, MAPK, p38, and NF-κB in vascular smooth muscle cells and the authors suggest that nucleotides may promote atherosclerosis via these mechanisms [42]. Vascular endothelial cells are continuously exposed to variations in blood flow, which plays an important role in vessel growth or regression and in the local development of atherosclerosis. The shear stress leads to a substantial release of ATP and UTP from endothelial cells [43], and these purines might mediate alterations in the balance between proliferation and apoptosis.

The pattern of vascular connexins is altered during atherosclerotic plaque formation and in restenosis affecting gap-junctional communication between smooth muscle cells, and genetically modified connexin expression alters the course of atherosclerosis and restenosis [44]. Connexin hemichannel formation has been shown to be regulated by ATP [45,46].

In restenosis following balloon angioplasty, there is a peak in the proliferation and apoptosis of vascular smooth muscle at about 14 days [47]. The first balloon inflation during coronary angioplasty is a preconditioning stimulus leading to a decrease in ischemia in later inflations; intracoronary adenosine administration before coronary angioplasty modifies the preconditioning effect of the first inflation [48].

Saphenous vein, internal mammary, and radial arteries have been used as grafts for coronary bypass surgery; the level of endothelial $P2Y_2$ receptors is comparable in all three vessels, but endothelial $P2X_4$ receptors vary from high in saphenous vein to significantly lower in the other two vessels. It has been suggested that $P2X_4$ receptors play a more significant role in intense proliferation in arteriosclerosis and restenosis than $P2Y_2$ receptors, as reflected by the susceptibility of saphenous vein grafts to atherosclerosis compared with internal mammary arteries [49]. In another study, $P2X_1$ and $P2Y_6$ receptors mediated more prominent contractions in the saphenous vein compared with the internal mammary artery; it has been suggested that selective antagonists to these receptors may prevent vasospasm and restenosis in the saphenous vein during and after revascularization surgery [50]. A novel role for $P2Y_2$ receptors in the development of atherosclerosis has been suggested, whereby UTP induces vascular cell adhesion molecule-1 expression in coronary artery endothelial cells that mediate the recruitment of monocytes [51]. Deletion of ecto-5'-nucleotidase (CD73), which leads to increase in extracellular ATP and reduction of adenosine, is characterized by enhanced platelet activation and increased adherence of monocytes to the endothelium. It has been shown that CD73-derived adenosine acts as an endogenous modulator protecting against vascular inflammation and monocyte recruitment, thus limiting the progression of atherosclerosis [52]. A recent paper has shown that P2 receptors play an important role in homocysteine-induced atherosclerosis [53].

Newly developing vascular endothelia express very high levels of the ectonucleotidase, NTPDase1, also seen under hypoxic conditions [54]. Angiogenesis requires the dynamic interaction of endothelial cell proliferation and differentiation with orchestrated interactions between extracellular matrix and surrounding cells (such as vascular smooth muscle and/or pericytes). Such interactions could be coordinated by interplay between nucleotide release, P2 receptor modulation, and altered NTPDase expression [55]. Atherosclerosis is an inflammatory disease induced by hypercholesterolemia and increase in NTPDase (CD39, apyrase) and subsequent ATP and ADP hydrolysis has been shown in platelets of hypercholesteremic patients [56]. The authors suggest that this might be beneficial in reducing thrombus formation, but may also contribute to future fatal events such as unstable angina and myocardial infarction.

In conclusion, the long-term (trophic) roles of purinergic signaling in vascular smooth muscle and endothelial cell proliferation and death have been implicated in atherosclerosis and restenosis and suggest the exploration of therapeutic strategies in relation to these events [3,17,57-61].

References

1. Burnstock G. 1972. Purinergic nerves. *Pharmacol Rev* 24: 509-81.
2. Burnstock G. 1997. The past, present and future of purine nucleotides as signalling molecules. *Neuropharmacology* 36: 1127-39.
3. Burnstock G. 2006. Pathophysiology and therapeutic potential of purinergic signaling. *Pharmacol Rev* 58: 58-86.
4. Abbracchio MP, Williams M. 2001. Eds. *Handbook of experimental pharmacology: purinergic and pyrimidinergic signalling*. Berlin: Springer.

5. Schwiebert EM, Zsembery A, Geibel JP. 2003. Cellular mechanisms and physiology of nucleotide and nucleoside release from cells: current knowledge, novel assays to detect purinergic agonists, and future directions. *Current Topics in Membranes* 54: 31-58.
6. Ralevic V, Burnstock G. 1998. Receptors for purines and pyrimidines. *Pharmacol Rev* 50: 413-92.
7. North RA. 2002. Molecular physiology of P2X receptors. *Physiol Rev* 82: 1013-67.
8. Burnstock G. 2004. Introduction: P2 receptors. *Curr Top Med Chem* 4: 793-803.
9. Abbracchio MP, Burnstock G, Boeynaems J-M, et al. 2006. International Union of Pharmacology. Update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. *Pharmacol Rev* 58: 281-341.
10. Abbracchio MP, Burnstock G. 1998. Purinergic signalling: pathophysiological roles. *Jpn J Pharmacol* 78: 113-45.
11. Burnstock G. 2006. Purinergic signalling - an overview. Novartis Foundation Symposium 276 Purinergic signalling in neuron-glial interactions. Chichester: John Wiley & Sons, Ltd., 26-53.
12. Hou M, Malmsjo M, Moller S, et al. 1999. Increase in cardiac $P2X_1$- and $P2Y_2$-receptor mRNA levels in congestive heart failure. *Life Sci* 65: 1195-206.
13. Hou M, Moller S, Edvinsson L, Erlinge D. 2000. Cytokines induce upregulation of vascular $P2Y_2$ receptors and increased mitogenic responses to UTP and ATP. *Arterioscler Thromb Vasc Biol* 20: 2064-69.
14. Burnstock G. 2002. Purinergic signalling and vascular cell proliferation and death. *Arterioscler Thromb Vasc Biol* 22: 364-73.
15. Burnstock G. 2006. Vessel tone and remodeling. *Nature Medicine - News and Views* 12: 16-17.
16. Burnstock G. 2008. Dual control of vascular tone and remodelling by ATP released from nerves and endothelial cells. *Pharmacol Rep* 60: 12-20.
17. Ralevic V, Burnstock G. 2003. Involvement of purinergic signalling in cardiovascular diseases. *Drug News Perspect* 16: 133-40.
18. Yamamoto K, Sokabe T, Matsumoto T, et al. 2006. Impaired flow-dependent control of vascular tone and remodeling in $P2X_4$-deficient mice. *Nat Med* 12: 133-37.
19. Jankowski V, Tölle M, Vanholder R, et al. 2005. Uridine adenosine tetraphosphate: a novel endothelium- derived vasoconstrictive factor. *Nat Med* 11: 223-27.
20. Gui Y, Walsh MP, Jankowski V, Jankowski J, Zheng XL. 2008. Up_4A stimulates endothelium-independent contraction of isolated rat pulmonary artery. *Am J Physiol Lung Cell Mol Physiol* 294: L733-L738.
21. Di Virgilio F, Solini A. 2002. P2 receptors: new potential players in atherosclerosis. *Br J Pharmacol* 135: 831-42.
22. Plank MJ, Wall DJ, David T. 2006. Atherosclerosis and calcium signalling in endothelial cells. *Prog Biophys Mol Biol* 91: 287-313.
23. Plank MJ, Wall DJ, David T. 2007. The role of endothelial calcium and nitric oxide in the localisation of atherosclerosis. *Math Biosci* 207: 26-39.
24. Seye CI, Kong Q, Yu N, Gonzalez FA, Erb L, Weisman GA. 2006. P2 receptors in atherosclerosis and postangioplasty restenosis. *Purinergic Signal* 2: 471-80.
25. Jonzon B, Nilsson J, Fredholm BB. 1985. Adenosine receptor-mediated changes in cyclic AMP production and DNA synthesis in cultured arterial smooth muscle cells. *J Cell Physiol* 124: 451-56.
26. Adair TH. 2005. Growth regulation of the vascular system: an emerging role for adenosine. *Am J Physiol Regul Integr Comp Physiol* 289: R283-R296.
27. Dubey RK, Gillespie DG, Jackson EK. 1999. Adenosine inhibits collagen and total protein synthesis in vascular smooth muscle cells. *Hypertension* 33: 190-94.
28. Wang DJ, Huang NN, Heppel LA. 1992. Extracellular ATP and ADP stimulate proliferation of porcine aortic smooth muscle cells. *J Cell Physiol* 153: 221-33.

29. Erlinge D, You J, Wahlestedt C, Edvinsson L. 1995. Characterisation of an ATP receptor mediating mitogenesis in vascular smooth muscle cells. *Eur J Pharmacol* 289: 135-49.
30. Malam-Souley R, Seye C, Gadeau AP, et al. 1996. Nucleotide receptor P2u partially mediates ATP-induced cell cycle progression of aortic smooth muscle cells. *J Cell Physiol* 166: 57-65.
31. Seye CI, Kong Q, Erb L, et al. 2002. Functional $P2Y_2$ nucleotide receptors mediate uridine 5'-triphosphate-induced intimal hyperplasia in collared rabbit carotid arteries. *Circulation* 106: 2720-26.
32. Carpenter RC, Miao L, Miyagi Y, Bengten E, Zhang JH. 2001. Altered expression of P2 receptor mRNAs in the basilar artery in a rat double hemorrhage model. *Stroke* 32: 516-22.
33. Hill BJ, Wamhoff BR, Sturek M. 2001. Functional nucleotide receptor expression and sarcoplasmic reticulum morphology in dedifferentiated porcine coronary smooth muscle cells. *J Vasc Res* 38: 432-43.
34. Elmaleh DR, Narula J, Babich JW, et al. 1998. Rapid noninvasive detection of experimental atherosclerotic lesions with novel 99mTc-labeled diadenosine tetraphosphates. *Proc Natl Acad Sci U S A* 95: 691-95.
35. McAuslan BR, Reilly WG, Hannan GN, Gole GA. 1983. Angiogenic factors and their assay: activity of formyl methionyl leucyl phenylalanine, adenosine diphosphate, heparin, copper, and bovine endothelium stimulating factor. *Microvasc Res* 26: 323-38.
36. Van Coevorden A, Roger P, Boeynaems J-M. 1989. Mitogenic action of adenine nucleotides and nucleosides on aortic endothelial cells. *Thromb Haemost* 62: 190.
37. Bodin P, Burnstock G. 1998. Increased release of ATP from endothelial cells during acute inflammation. *Inflamm Res* 47: 351-54.
38. Gorini S, Mammi C, Ferrari D, et al. 2007. Abstract 134: Extracellular ATP inhibits natural killer cell recruitment in inflamed tissues and protects endothelial cells from NK-mediated vascular injury. *Circulation* 116: II.
39. Thomas WA, Reiner JM, Florentin FA, Lee KT, Lee WM. 1976. Population dynamics of arterial smooth muscle cells. V. Cell proliferation and cell death during initial 3 months in atherosclerotic lesions induced in swine by hypercholesterolemic diet and intimal trauma. *Exp Mol Pathol* 24: 360-74.
40. Mallat Z, Tedgui A. 2000. Apoptosis in the vasculature: mechanisms and functional importance. *Br J Pharmacol* 130: 947-62.
41. Brambilla R, Burnstock G, Bonazzi A, Ceruti S, Cattabeni F, Abbracchio MP. 1999. Cyclo-oxygenase-2 mediates P2Y receptor-induced reactive astrogliosis. *Br J Pharmacol* 126: 563-67.
42. Wang W-J, Yang C-M. 2006. ATP-induced COX-2 expression via p42/p44 MAPK, p38, and NF-kB in vascular smooth muscle cells. *FASEB J* 20: A229-A22a.
43. Burnstock G. 1999. Release of vasoactive substances from endothelial cells by shear stress and purinergic mechanosensory transduction. *J Anat* 194: 335-42.
44. Chadjichristos CE, Derouette JP, Kwak BR. 2006. Connexins in atherosclerosis. *Adv Cardiol* 42: 255-67.
45. Jiang L, Bardini M, Keogh A, dos Remedios CG, Burnstock G. 2005. $P2X_1$ receptors are closely associated with connexin 43 in human ventricular myocardium. *Int J Cardiol* 98: 291-97.
46. Lurtz MM, Louis CF. 2007. Purinergic receptor-mediated regulation of lens connexin43. *Invest Ophthalmol Vis Sci* 48: 4177-86.
47. Han DK, Haudenschild CC, Hong MK, Tinkle BT, Leon MB, Liau G. 1995. Evidence for apoptosis in human atherogenesis and in a rat vascular injury model. *Am J Pathol* 147: 267-77.
48. Kerensky RA, Kutcher MA, Braden GA, Applegate RJ, Solis GA, Little WC. 1995. The effects of intracoronary adenosine on preconditioning during coronary angioplasty. *Clin Cardiol* 18: 91-96.
49. Ray FR, Huang W, Slater M, Barden JA. 2002. Purinergic receptor distribution in endothelial cells in blood vessels: a basis for selection of coronary artery grafts. *Atherosclerosis* 162: 55-61.

50. Borna C, Wang L, Gudbjartsson T, et al. 2003. Contractions in human coronary bypass vessels stimulated by extracellular nucleotides. *Ann Thorac Surg* 76: 50-57.
51. Seye CI, Yu N, Jain R, et al. 2003. The $P2Y_2$ nucleotide receptor mediates UTP-induced vascular cell adhesion molecule-1 expression in coronary artery endothelial cells. *J Biol Chem* 278: 24960-24965.
52. Buchheiser A, Emde B, Fischer JW, Schrader J. 2007. Abstract 623: Role Of Cd73-derived adenosine in atherosclerosis. *Circulation* 116: II.
53. Sharma M, Rai SK, Tiwari M, Chandra R. 2007. Effect of hyperhomocysteinemia on cardiovascular risk factors and initiation of atherosclerosis in Wistar rats. *Eur J Pharmacol* 574: 49-60.
54. Eltzschig HK, Ibla JC, Furuta GT, et al. 2003. Coordinated adenine nucleotide phosphohydrolysis and nucleoside signaling in posthypoxic endothelium: role of ectonucleotidases and adenosine A_{2B} receptors. *J Exp Med* 198: 783-96.
55. Goepfert C, Sundberg C, Sevigny J, et al. 2001. Disordered cellular migration and angiogenesis in cd39-null mice. *Circulation* 104: 3109-15.
56. Duarte MM, Loro VL, Rocha JB, et al. 2007. Enzymes that hydrolyze adenine nucleotides of patients with hypercholesterolemia and inflammatory processes. *FEBS J* 274: 2707-14.
57. Erlinge D. 1998. Extracellular ATP: a growth factor for vascular smooth muscle cells. *Gen Pharmacol* 31: 1-8.
58. Burnstock G. 2002. Potential therapeutic targets in the rapidly expanding field of purinergic signalling. *Clin Med* 2: 45-53.
59. Hou M, Harden TK, Kuhn CM, et al. 2002. UDP acts as a growth factor for vascular smooth muscle cells by activation of $P2Y_6$ receptors. *Am J Physiol Heart Circ Physiol* 282: H784-H792.
60. Wang L, Andersson M, Karlsson L, et al. 2003. Increased mitogenic and decreased contractile P2 receptors in smooth muscle cells by shear stress in human vessels with intact endothelium. *Arterioscler Thromb Vasc Biol* 23: 1370-76.
61. Erlinge D, Burnstock G. 2008. P2 receptors in cardiovascular physiology and disease. *Purinergic Signal* 4: 1-20.

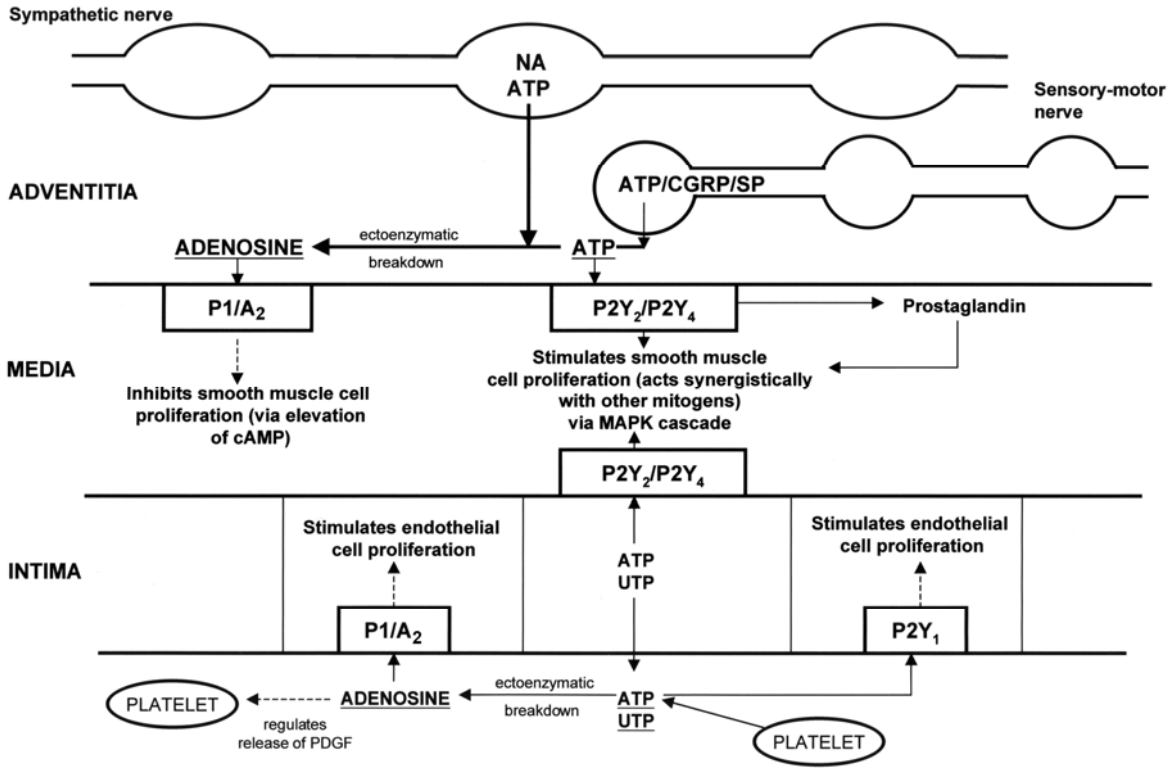

Figure 1. Schematic diagram of long-term (trophic) actions of purines released from nerves, platelets, and endothelial cells (which also release UTP) acting on P2 receptors to stimulate or inhibit cell proliferation. ATP released as a cotransmitter from sympathetic nerves and sensory-motor nerves (during axon reflex activity) stimulates smooth muscle cell proliferation via $P2Y_2$ and/or $P2Y_4$ receptors via a mitogen activated protein kinase (MAPK) cascade, whereas adenosine resulting from enzymatic breakdown of ATP acts on P1 (A_2) receptors to inhibit cell proliferation (via elevation of cAMP). ATP and UTP released from endothelial cells stimulate endothelial and smooth muscle cell proliferation via $P2Y_1$, $P2Y_2$ and $P2Y_4$ receptors. Adenosine resulting from ATP breakdown acts on P1 (A_2) receptors to stimulate endothelial cell proliferation and regulate the release of platelet-derived growth factor (PDGF) from platelets. NA, noradrenaline; CGRP, calcitonin gene-related peptide; SP, substance P. (Reproduced from [14] with permission from Lippincott, Williams and Wilkins).