# Ultrasound and Autism: How Disrupted Redox Homeostasis and Transient Membrane Porosity Confer Risk

**Emily L. Williams and Manuel F. Casanova**

## Abbreviations

| | |
|---|---|
| AP-1 | Activator protein 1 |
| APC | Activated protein C |
| ATP | Adenosine triphosphate |
| cAMP | Cyclic adenosine monophosphate |
| cGMP | Cyclic guanosine monophosphate |
| CRE | cAMP response element |
| CREB | cAMP response element binding protein |
| CSF | Cerebrospinal fluid |
| ERK | Extracellular signal-regulated protein kinase |
| FDA | United States Food and Drug Administration |
| FGF | Fibroblast growth factor |
| GMP | Guanosine monophosphate |
| GSK3β | Glycogen synthase kinase 3β |
| HAT | Histone acetyltransferases |
| HDAC | Histone deacetylase |
| iNOS | Irreducible nitric oxide synthase |
| JNK | c-Jun N-terminal kinase |

E.L. Williams, Ph.D. (✉)
Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, 500 South Preston Street, HSC-A906, Louisville, KY 40202, USA
e-mail: elwill08@louisville.edu

M.F. Casanova, M.D.
Department of Psychiatry and Behavioral Sciences, University of Louisville School of Medicine, Louisville, KY 40202, USA
e-mail: manuel.casanova@louisville.edu

© Springer Science+Business Media New York 2015

A. Dietrich-Muszalska et al. (eds.), *Studies on Psychiatric Disorders*,
Oxidative Stress in Applied Basic Research and Clinical Practice,
DOI 10.1007/978-1-4939-0440-2_18

| | |
|---|---|
| LAMP-1 | Lysosome-associated membrane glycoprotein 1 |
| LEF | Lymphoid enhancer factor |
| LRP | Lipoprotein receptor-related protein |
| LTP | Long-term potentiation |
| MAPK | Mitogen-activated protein kinase |
| mTOR | Mammalian target of rapamycin |
| NFκB | Nuclear factor κB |
| NMDA | N-methyl-D-aspartate |
| $PIP_2$ | Phosphatidylinositol (4,5)-bisphosphate |
| $PIP_3$ | Phosphatidylinositol (3,4,5)-trisphosphate |
| PKA | Protein kinase A |
| PKC | Protein kinase C |
| PTEN | Phosphatase and tensin homologue |
| redox | Reduction-oxidation |
| ROS | Reactive oxygen species |
| TCF | T cell factor |
| US | Ultrasound |

## 1 Introduction

The study of sonic waveforms long predates the invention and application of the current forms of clinical ultrasound (US). As early as 1826, Jean-Daniel Colladon, a Swiss physicist and engineer, and Charles-Francois Sturm, a mathematician, studied hydroacoustics by utilizing the combination of an underwater bell and a simultaneous ignition of gunpowder to compare speed of underwater sound to that of light, thereby estimating the former at 1,435 m/s. While this early study of sonification was of an academic vein, underwater sonar was eventually studied, refined, and popularized for submarine navigation during World War I. The disastrous voyage of the *Titanic* likewise highlighted the need for better means of navigation. By the 1930s, ultrasound had been exapted for such uses as radar and metal flaw detection, the latter largely utilized for detecting flaws within the metal construction in hulls of large ships and plates of battle tanks. Also by this time, high-intensity ultrasound was being recognized as a useful therapeutic tool by the medical industry. Eventually, it evolved into an important tool for neurosurgery. Unfortunately, the 1940s saw an uprise in enthusiasm for ultrasound use, leading to boisterous claims of its cure-all capacity and its utilization in a variety of unwarranted therapies. Simultaneously, a mounting skepticism grew – a skepticism which ultimately retarded research into ultrasound's numerous applications, such as diagnostics.

Following a body of research investigating its bioeffects, ultrasound has slowly overcome the medical community's initial skepticism from the 1940s to 1950s. However, a similar albeit more confined overconfidence in ultrasound's safety has led to its overuse within obstetrics. For the first few decades of ultrasound's use in prenatal care, the FDA strictly regulated absolute intensity levels according to the

specific application. Now, however, risk is assessed by real-time thermal and cavitational indices available on the device itself. This has shifted control away from a regulatory authority dictating absolute exposure levels, to the end user who interprets machine output and adapts usage based on medical experience (Barnett et al. 2000).

To complicate matters, machine reliability is being called into question. Recently, a series of studies assessing ultrasound transducer error rates reveals that of seven manufacturer's equipment tested across 676 different transducers, on average 40 % of those transducers were defective (Mårtensson et al. 2009). All companies tested exhibited a minimum of 20 % error rates, while one company tested as high as 67 %. A separate study by the same research group sampled additional ultrasound transducers in a single hospital setting, finding 81 of 299 actively used ultrasound machines to be defective. Approximately 83 % of these errors were due to delamination of the ultrasound lens or breaks in the cables (Mårtensson et al. 2010). The group stresses the need for thorough and frequent transducer testing, beyond that of annual testing, in part because it is usually very difficult for the sonographer to recognize transducer defects due to a slow degradation of image quality that may not be readily identifiable. However, not only for the purpose of image quality and diagnostics is it imperative that ultrasound machines perform as they are intended: faulty transducers lead one to question whether the safety indices are accurately gauging exposure levels and, if not, what the true range of exposure levels may be. With faulty equipment, there is no way to ensure that exposures are not reaching harmful ranges.

In addition to questionable transducer performance, practitioners and sonographers who routinely utilize ultrasound in their practices appear to be poorly educated in the possible risks of ultrasound exposure. In a survey of 130 end users, 82.3 % failed to demonstrate understanding of the term "thermal index" which gauges temperature levels within exposed tissue, 96.2 % failed to demonstrate understanding of "mechanical index" which gauges cavitational levels within the tissue, and, alarmingly, only 20 % of end users knew where these safety indices were located on the machines (Sheiner et al. 2007). While patient safety is now dependent on machine performance and sonographer judgment, it is clear that earlier FDA deregulations have placed the onus of that safety on the shoulders of faulty equipment and undereducated end users.

Along with deregulated medical practices, ultrasound services are available for private use through businesses which offer additional ultrasonography so that patrons can "start the family photo album early," have 3D image renderings of the baby, and even have videos made. Ultrasound equipment is also available for purchase at websites such as eBay and Amazon, and additional devices like Doppler heart rate monitors can be purchased for daily, medically unsupervised monitoring of the baby's heartbeat. While the FDA has warned against these services and the use of such devices within the home, these warnings have been poorly publicized and the vast majority of the public and practitioners are unaware of their potential dangers.

However, not only are there poor regulations, poor practitioner and public education, and faulty equipment; the understanding of the basic biophysics of ultrasound has been weakly applied across disciplines. There is a considerable body of evidence which illustrates ultrasound's bioeffects on various cell and tissue types; however,

when reviewing the literature, it appears that little of this knowledge has been viewed in light of prenatal care. Instead, articles abound on cardiovascular studies, targeted drug therapy, transmembrane delivery of nonviral genes, and even transcranial stimulation of brain circuitry – each of these illustrating a host of its primary and secondary effects on the cell. It is amazing that prior to now, there has been only a minimal application of this vast body of knowledge to the study of ultrasound exposure and prenatal development (for some of the few examples in the literature, see Dinno et al. 1989; Ang et al. 2006; Schneider-Kolsky et al. 2009).

With the climbing rates of autism, the increasing frequency of ultrasound use and its frightening deregulations, and what we currently know about the basic biophysics of ultrasound, links are being drawn between cellular morphologies characteristic of autism and those which result from ultrasonic exposure. In addition, there are superficial links between their various cell metabolics, including altered rates of reduction-oxidation (redox) reactions at lipid membranes as well as throughout the cell, abnormalities in calcium signaling, upregulation of certain growth factor signaling pathways, and even modifications in key epigenetic patterns. One likely reason why these bioeffects have rarely been studied following prenatal ultrasound is because they are subtle, usually microscopic, and may involve changes in transcription and not the degradation of tissues or the mutagenesis of DNA. It takes the skilled study of microscopic morphology and shifts in molecular networks to gauge how ultrasound functions as a teratogen. Ultimately, there may be some avoidance within the medical and lay communities to disturb the status quo: not only do parents want that first picture of their unborn child, but ultrasound as an industry makes good money. Should prenatal ultrasound ultimately prove unsafe in its current application, it will take the cooperation of research, medicine, business, government, and the public to tighten regulations and ensure, as useful a tool as it is, that the risks do not outweigh the benefits.

## 2 Two Main Effectors: Transient Membrane Porosity and Disruption of Redox Homeostasis

While most laymen think of obstetric ultrasound as an image, there are distinct differences between sonography and photography. The medium of photography for instance is light or photons; light enters the aperture of the camera and is then recorded on film or an electronic image sensor. While flash bulbs are often employed, from the point of view of the camera, this entire process is fairly passive: the camera measures the dispersed rays of light already present within the environment. An ultrasound, however, not only captures the sound vibrations of a target object; it also actively creates them and focuses them onto a target. Therefore, unlike a camera, the ultrasound is aiming sound waves onto an object then measuring the echo as the sound returns to the device.

Scientific convention has normally separated ultrasound mechanics into thermal and nonthermal means, the latter comprising cavitational and noncavitational mechanisms. Noncavitational mechanisms include forces such as radiation torque, radiation

force, radiation pressure, and acoustic streaming, all reflective of the different types of fluid pressure placed on the cell surface by the ultrasound beam. Cavitation, named for the gaseous cavities induced by ultrasound exposure, includes two subtypes: stable and transient. The former denotes bubbles which have formed; oscillate, creating a shear stress (stress which is applied parallel or tangential to a surface); and remain intact during many acoustic cycles. The latter, transient cavitation, refers to the formation, expansion, and implosion of bubbles during a compression half cycle from which a shock wave of pressure is emitted. This shock wave can rupture the cellular membrane, triggering necrosis or apoptotic pathways, or, at lower intensities, can create transient porosity of the membrane leading to teratogenic yet nonlethal chains of events (Riesz and Kondo 1992, for review).

Though conventional science and medicine have been most concerned with risks of hyperthermia and tissue damage due to shock waves from transient cavitation, we would like to broaden the research and medical communities' focus to include less deleterious but no less teratogenic mechanisms: namely, the transient permeation of the membrane and the redox reactivity resultant from lower intensity cavitation. This includes both cavitational and noncavitational means in the application of mechanical force upon the cell, as well as free radical release from the imploded cavity. It is these elements, we argue, which have the most teratogenic potential because they do not endanger the cell but alter genetic expression and epigenetic patterning, potentially leading to multigenerational cellular effects from a single exposure. For instance, Liebeskind et al. (1981) found that cultured cells expressed changes in their overall morphology following ultrasound exposure, exhibiting abundant numbers of irregular microvilli which are small pseudopod extensions arising from the surface of the cell due to actin polymerization (or growth) of the actin cytoskeleton. These were present as early as three days following exposure and were still present 37 days later, suggesting that ultrasound had led to permanent changes in DNA transcription. Ultrasound may be capable of inducing this actin polymerization via targeting the MAPK pathway, possibly through a series of events triggered by hyperosmotic shock, through redox-triggered phosphorylation of MAPK, and also through direct targeting of the redox-sensitive cytoskeleton itself (Ho 2006; Zhu et al. 2005; Fiaschi et al. 2006; McDonagh et al. 2005).

As mentioned, increased membrane permeability is one of two important biological effectors in ultrasound exposure. Upon exposure, minute gaseous bubbles present in the fluids surrounding and within the cell begin to grow through a process called rectified diffusion, oscillate, and then implode, creating shock waves that assault the cell membrane. Cavities which do not implode but remain for several cycles create shear stress across the membrane. Likewise, noncavitational means can increase membrane permeability simply through radiative forces of water against the cell. When the water molecules comprise large enough clusters, the lipid membrane is disturbed, creating a pore of approximately 1.4 nm in diameter (Minkel 2010, for review). These pores then reseal following the influx of calcium, which triggers fusion of lysosomes with the membrane in a lysosomal-associated membrane protein 1 LAMP-1-dependent manner (Deng et al. 2004; Reddy et al. 2001; Yang et al. 2008). During this transient porosity, a portion of extracellular fluids is taken up into

the cell, triggering a cascade of molecular activity in response to the breakdown of barriers between intracellular and extracellular compartments. To give some perspective, gap junctions, which are direct open funnels between select cells and allow the passage of larger molecules between those cells, are approximately 1.2–2 nm in diameter (Hormuzdi et al. 2004; Bennett and Zukin 2004). This is very similar in size to the 1.4 nm transient pore that is created during ultrasound exposure from noncavitational radiative forces. Such a pore would allow the rapid influx of ions like calcium and sodium into the cell, as well as other external amino acids and cellular metabolites, and the efflux of ions such as potassium (Pébay et al. 2011, for review). This in turn would trigger chains of events related to the breakdown of barriers, such as conduction within neurons, and downstream targeting of CREB, PKC, MAPK, cAMP, cGMP, histone deacetylases (HDACs), and numerous other pathways. For pathways which function as positive feedback loops, or which lead to alterations in epigenetic patterns such as the targeting of HDACs, such transient permeability may permanently alter the phenotype of the cell. In addition, the gap junctions which are vital in the development and maintenance of various stem cell populations may further increase tissue reactivity to transient prenatal exposure by sharing these influxes among all interconnected cells, and in support of these, studies have noted the occurrence of calcium waves during and following ultrasound (Kumon et al. 2007).

Prior to the realization that water pressure alone could alter the fluidity and permeability of cellular membranes, it was considered that the redox reactions, or lipid peroxidation, in the outer membrane might cause the degradation seen therein (e.g., see Juffermans et al. 2006). Under intense redox conditions, this may be possible; however, groups such as Lawrie et al. (2003) have found that the uptake and subsequent expression of nonviral transgenes are not dependent upon redox mechanisms but utilize another means of entry. Koshiyama et al. (2006) found that structural changes of the phospholipid bilayer due to ultrasound-induced jets of water were enough to create transient pores in the membrane. While lipid peroxidation can indeed alter the fluidity and subsequent permeability of the cell, the more immediate effector of porosity appears to be the radiative force of water on the phospholipid bilayer (Zhu et al. 2005; Pohl et al. 1993; Koshiyama et al. 2006).

Redox reactions are, however, responsible for a number of the bioeffects seen following clinical ultrasound and are therefore considered here as a second main effector of exposure. *Redox*, short for reduction-oxidation, refers to the theft of an electron by a free radical from a surrounding compound or molecule, with a new free radical forming in its place. This chain reaction continues like a string of dominoes and can be thousands of reactions long. Unsaturated fats, particularly polyunsaturated fats, within the lipid membrane can be especially vulnerable to this kind of attack due to the weak double-carbon bonds present in these unsaturated lipids. A reactive oxygen species (ROS) can target the multiple carbon double bonds within the polyunsaturated fatty acid, allowing for easy dissociation of the hydrogen atoms present in the lipid. Another free radical can then steal the unpaired electron from the hydrogen linked with the carbon double bond, turning the carbon into a free radical itself. This conjugated diene can then react with oxygen to form a proxy radical, which thus propagates the redox chain reaction, until at which point two free radicals meet and form a covalent bond with one another. Mitochondrial membranes are

likewise vulnerable to redox mechanisms, not only from the surrounding environment but from within the mitochondrion itself through the production of ATP which produces superoxide anions as a by-product. Superoxide can then either be turned into hydrogen peroxide through the intervention of superoxide dismutase or can form a highly reactive ROS, a hydroxyl radical. Proteins, RNA, and DNA are also targets of oxidation; however, they are comparatively less vulnerable than the polyunsaturated lipids in the various cellular membranes, not only due to their molecular composition but also because of their locations within the cell, given that membranes generally feel the first brunt of a free radical attack.

The sonolysis of water produces hydroxyl radicals ($OH^-$) as one of its main by-products. Of the initial molecular products succeeding this reaction is the formation of hydrogen peroxide ($H_2O_2$), hydrogen atoms, water, and other oxidized molecules coming from the cellular landscape, such as peroxidized lipids (for review, see Riesz and Kondo 1992). Increased levels of hydrogen peroxide have also been noted in vivo following ultrasound exposure (Juffermans et al. 2006). The production of this and other reactive oxygen species, such as the aforementioned conjugated dienes, lipid hydroperoxides, and malondialdehydes, each exhibits a dose-dependent increase following ultrasound. This in turn is tightly linked with the occurrence of lipid peroxidation within the membrane (Jana et al. 1990). Following exposure, there are changes in membrane fluidity as well as changes in cytoskeletal arrangement mentioned previously (Zhu et al. 2005). Actin polymerization may be triggered through a number of means, each working in concert to create the microvillus extensions seen following ultrasound. The MAPK pathway is a prime potential target for ultrasound-induced oxidation, given its natural sensitivity to redox mechanisms under normal circumstances. It has been shown that if MAPK is inhibited during $H_2O_2$ exposure, the membrane and cytoskeletal changes reminiscent of ultrasonic exposure which would have otherwise resulted will instead be suppressed (Zhu et al. 2005).

As mentioned, the cytoskeleton is itself a direct target of redox reactions. While oxidation is normally considered as a destructive mechanism, actin oxidation is necessary for the growth of the cytoskeleton (Fiaschi et al. 2006; McDonagh et al. 2005). Cell-to-cell adhesion, however, is also a necessary component in the maintenance of stem cell fate, and loss of that adhesion, such as occurs following oxidation-induced polymerization of the cytoskeleton, can cause cells to differentiate prematurely, creating heterochronies in tissue development (Campos et al. 2004). It has been shown that following US exposure, such loss of adhesion can occur (e.g., see Siegel et al. 1979). For neural stem cell fate, this can alter subpopulation numbers of mature cells and thus lead to changes within the structure and function of the entire cerebral system.

## *2.1 Secondary Effects Following US Exposure*

When discussing secondary effects following low-level ultrasound exposure, one must ultimately ask: What are the general downstream effects of transient membrane porosity and the production of free radicals? A US-induced membrane pore is

similar in diameter to that of a gap junction and allows the influx and efflux of ions, amino acids, and other metabolites which would also flow through gap junctions. In this instance, however, extracellular contents are allowed into the cell while intracellular contents are allowed out, different from a gap junction which allows the exchange of intracellular fluids between cells.

In the case of ions, the creation of a transient pore within the membrane is indiscriminate and the equivalent of briefly opening all ionic channels at once. Sodium, calcium, and chlorine rush into the cell, and potassium rushes out altering their concentration gradients. Electrochemically, this alters the resting membrane potential of the cell and can lead to polarization and thus action potentials (Tyler et al. 2008). In the central nervous system, this triggers chains of events related to vesicular release of neurotransmitters into the synapse, as well as intracellular chains of events following calcium influx, such as signal transduction through G protein-coupled receptors. Ultimately, this activation can lead to events such as neuronal transmission, long-term potentiation (LTP) through the NMDA receptor, and motility, growth, and proliferation in the case of immature cells (Berridge et al. 2000). As can be deduced by these chains of events, ultrasound leads to calcium influx, and calcium influx can subsequently lead to changes in gene expression.

Calcium influx ensues transient membrane porosity. As discussed, a continuation of calcium waves generally follows this permeability, targeting a host of downstream effectors (Kumon et al. 2007). In neural stem cells and progenitors, calcium waves appear to control their proliferation. Transient increases in calcium can also lead to cell differentiation and neuritogenesis of these populations (Pébay et al. 2011). As an intracellular signaling molecule, calcium targets pathways such as MAPK/ERK, calmodulin, and PKA, which all converge onto CREB. CREB then binds DNA sequences called *cAMP response elements* (*CRE*) and, depending on its binding partners, can either agonize or antagonize expression of genes such as *c-fos*, *BDNF*, *corticotropin-releasing hormone, tyrosine hydroxylase*, and *enkephalin* (Zhang et al. 2005). Some of the calcium receptors are likewise vulnerable to redox regulation, having a so-called redox switch in which oxidative mechanisms turn the receptor "on," whereas reduction turns the receptor "off" regardless of upstream activation (Campbell et al. 1996).

As one can see as per example of the calcium receptor, redox-induced chains of events can lead to considerable alterations in phenotypic expression. This is because redox mechanisms regulate many of the same pathways which are triggered by changes in resting membrane potential as seen following transient porosity, such as pathways for cell survival, growth, proliferation, motility, and even apoptosis. In pyramidal neurons, redox mechanisms also regulate NMDA receptor activity through the receptor's various sulfhydryl groups (Lei et al. 1992). Targets under direct or indirect regulatory control from reduction-oxidation include growth factors such as VEGF and EGF; cytokines such as TNF-α, IL-1β, and IL-8; various adhesion molecules such as integrins; the MAPK/ERK pathway; the Akt pathway; the canonical Wnt pathway; the JNK pathway; the NFκB pathways; AP-1; matrix metalloproteinases; the calcium and NMDA receptors mentioned before; and many others (Roy et al. 2008; Pébay et al. 2011; Rahman et al. 2004; Funato et al. 2006;

Buhimschi et al. 2000). Because redox mechanisms are so heavily influenced by environmental effectors, such as food intake, air quality, radiation exposure, and infection, it is speculated that reactivity to reduction-oxidation, particularly by integrating redox mechanisms into its cell signaling pathways, is one way the living organism adapts to the demands of its environment.

Previous studies have found that redox mechanisms regulate proliferation in numerous cell types (Ranjan et al. 2006; Griendling and Ushio-Fukai 1998). Likewise, ultrasound exposure itself has been shown to increase proliferation in populations of fibroblasts, osteoblasts, monocytes, chondrocytes, and endothelial progenitor cells (Doan et al. 1999; Zhang et al. 2003; Young and Dyson 1990a). Unsurprisingly, downstream of redox activity are targets such as *N-Myc*, a gene which acts both as a transcription factor but more importantly as a regulator for a large number of genes by recruiting histone acetyltransferases (HATs) to the DNA, thus altering chromatin structure and the epigenome (Medina et al. 1992; Cotterman et al. 2008). Oxidative stress is also capable of inhibiting histone deacetylase (HDAC) activity (Rahman et al. 2004). Because the hormone, glucocorticoid, functions to recruit HDACs to the site of gene expression, inhibiting HDACs thereby renders the cell insensitive to glucocorticoid activity and vulnerable to chronic inflammation (Adcock et al. 2005).

The inhibition of HDACs likewise can lead to the upregulation of the canonical Wnt pathway, thus leading to increased cellular proliferation (Wiltse 2005). The Wnt ligand binds to the LRP/Frizzled co-receptors which in turn activate the intracellular mediator, Dishevelled. Dishevelled then disrupts the Axin/APC complex which would normally bind and mark the transcription factor, β-catenin, for degradation; instead, β-catenin is freed into the cytosol, is then transported across the nuclear membrane, and subsequently binds and displaces Groucho, thereby activating LEF/TCF transcription and promoting cell cycle progression. If, however, an HDAC is bound to Groucho, this in turn prevents the binding of β-catenin to the complex and prevents cycle progression. The canonical Wnt pathway is subject to various redox controls: Dishevelled, for instance, is vulnerable to reduction mechanisms which suppress it (Funato et al. 2006); Wnt is also downstream from the Akt, mTOR, and ERK pathways through its involvement with GSK3β, and each of these pathways are redox regulated either via direct redox-induced phosphorylation or phosphorylation of one or more of their constituents (Murata et al. 2003; Sarbassov and Sabatini 2005). Given its various redox regulatory mechanisms, as well as its downstream position of pathways such as Akt, mTOR, and MAPK/ERK, it is perhaps unsurprising that Wnt's upregulation has been reported following ultrasound exposure (Olkku et al. 2010).

Ultrasound is known to upregulate production of a variety of products, such as cyclooxygenase-2, basic FGF, prostaglandin $E_2$, and nitric oxide (NO), although the precise cause, cavitational or noncavitational, is currently unknown (Hsu et al. 2007; Reher et al. 1999, 2002). While NO is certainly a free radical, its production in this instance is more likely due to one of transcription of inducible nitric oxide synthase (iNOS), based on the time course of its presentation following exposure, and not due directly to cavitation (Reher et al. 2002). Instead, its production is more

likely a result of activation of the MAPK/ERK and NFκB pathways (Hou et al. 2009). While NO normally acts as an antioxidant in its capacity to reduce lipid peroxyl radicals, its increased production does, however, raise the risk of itself forming a free radical, peroxynitrite, in the presence of superoxide anion which is produced by the electron transport chain (Hogg and Kalyanaraman 1999). While it can act as an antioxidant in its native form, NO also functions as an intracellular signaling molecule, such as activating the cyclic GMP pathway, and also plays important roles in vascular dilation which can subsequently affect the tissues around which the vasculature forms its niche (Shen et al. 2004).

## *2.2 Ultrasound and Corticogenic Heterochrony*

As with all tissues, the central nervous system follows a coordinated series of developmental events. The development of a given cell is determined not only by intrinsic factors but by extrinsic ones as well, particularly by temporal cues, location, and its orientation relative to the different cells surrounding it and its location in space. In relation to these cues, hydrodynamic forces play important roles in cellular instruction by helping to form chemical gradients. For instance, the flow of CSF within the ventricles, along with the help of ependymal cilia, promotes aspects of the rostral-caudal patterning of the cortex through a gradient distribution of key soluble instructive factors (Götz and Stricker 2006). Hydrodynamic forces and shear stress likewise play vital roles in the development and continued maintenance of the vascular system (Yamamoto et al. 2003), and the vasculature in turn forms a niche surrounding the neural stem cell population, releasing soluble factors which stimulate self-renewal, neurogenesis, and cell differentiation (Shen et al. 2004).

As one might imagine, ultrasound could impose a serious threat to cellular development by providing false navigational cues via hydrodynamics, cavitation, and reduction-oxidation mechanisms, leading to a heterochrony of neural tissues (for review, see Banerjee and Slack 2002; Guirao et al. 2010). In 2006, Ang et al. published work illustrating this phenomenon: mice were exposed in utero on embryonic day 16 (mid-to-late neurogenesis) to ultrasound, with exposure ranging anywhere from 5 to 420 min. Exposed and control mice were collected on postnatal day 10 and analyzed. The researchers found that following ultrasound, mouse cortex exhibited signs of migratory abnormalities as well as periventricular ectopias. While no behavioral studies were performed on these mice, Schneider-Kolsky et al. (2009) also exposed fetal chickens to 1–10 min of ultrasound and found that Doppler ultrasound in particular triggered significant memory and learning impairment in the range of 4–5 min of exposure. Doppler ultrasound is utilized to evaluate blood flow and is generally used in obstetrics for monitoring heart development; however, as a device it is also available for private use and can be purchased by expectant parents for unregulated use within the home.

The migratory and proliferative abnormalities seen in the US-exposed mouse cortices may have similar causal roots because both mitosis and cell motility utilize

overlapping molecular pathways to perform their functions. Pathways such as Akt, MAPK/ERK, mTOR, Wnt, and JNK exhibit a considerable cross talk and overlap of functions in proliferation and migration (for review, see Williams and Casanova 2011). For instance, PTEN is a protein which regulates the Akt pathway; when PTEN is downregulated, Akt is subsequently upregulated. In a *Pten* knockout model of autism and macrocephaly which specifically targets neural stems cells through a *Nestin-cre* combination, this mouse model not only exhibits drastically increased neurogenesis of certain subpopulations, but it also exhibits extreme laminar maldistribution, suggestive of migratory disturbances (Groszer et al. 2001). Mutations in the transcriptional repressors *Hes1*, *Hes3*, and *Hes5* cause combined abnormalities in proliferation and cell migration (Hatakeyama et al. 2004).

Heterochrony is a term used to describe not only variations in homologous tissues between species; it can also refer to variations within a species of a given tissue and whether cell fate is expressed comparatively early or is retarded in its development and remains in a state of immaturity for an extended period of time. Generally, this prolonged immaturity will involve continued symmetric or asymmetric proliferation and thus result in changes in ratios of cell subpopulations (Banerjee and Slack 2002, for review). On a broad scale, evolutionary theorists have proposed that the retarded heterochrony of the initial radial glial progenitor population has led to increases in encephalization across species, i.e., bigger brains. However, Casanova et al. (2002) also propose that such a heterochrony within the human population may underlie aspects of the autism phenotype, in which retarded development of radial glia leads to a greater number of neocortical minicolumns and a subsequent disturbance in overall cerebral connectivity patterns which cause the behavioral syndrome.

Such heterochronies can also be seen in the variety of autism animal models used to study the conditions. For instance, a model for Fragile X syndrome, a genetic condition highly comorbid with autism, exhibits a distinct increase in the number of Tbr1-immunolabeled cells within the infragranular layers of the neocortex, suggesting that the radial glia which produced these cells are held in a state of prolonged proliferation (Tervonen et al. 2009). The tuberous sclerosis model (*Tsc2* knockout), also highly comorbid with autism, conversely exhibits increased numbers of Tbr2-immunolabeled intermediate progenitors within the subventricular zone and a simultaneous decrease in numbers of infragranular cells, although total number of neurons within the cortex does not appear generally disturbed (Way et al. 2009). This model instead suggests that the *Tsc2* knockout's radial glia mature early, produce fewer infragranular neurons, and instead produce more intermediate progenitors, the latter which are subsequently maturationally retarded and held in a proliferative state for a longer period of time. Thus, both of these models display distinct heterochronies which lead to laminar anomalies different from one another and their controls and are reflective of the neocortical development seen in their respective human conditions, thus illustrating how slight changes in relative tissue development can drastically change the function of that tissue, e.g., behavior and cognition. Ultimately, just as with mutations in the *Fmr1* gene in Fragile X or in the *Tsc2* gene in Tuberous sclerosis, ultrasound may not play a teratogenic role in all cases of autism, but it may play an integral one in a portion of them, particularly

individuals who may be more susceptible to its effects either through inherited proclivity, due to differences in exposure, to multiple environmental hits from other agencies, or a combination thereof. In lieu of developmental plasticity and the capacity for development to be guided towards a particular common phenotype through different yet converging pathways, one can see how the molecular activities which result from ultrasound exposure may share commonalities with other causal factors already known to be associated with autism.

## *2.3 Factors Associated with Autism*

Abnormalities in cerebral growth have been a recognized association with autism for a number of years, in particular the increase in head circumference during childhood which generally normalizes by puberty or adulthood (Aylward et al. 2002). Casanova (2004) has noted that this initiation of unusual growth coincides with the time period for myelination, a process which is known to add considerable volume to the cerebral cortex. Instead, he suggests that this increase in volume is due to myelination and is reflective of the earlier corticogenic disturbances which ultimately lead to a rise in total cell numbers within the neocortex and subcortical structures. This retarded growth of the progenitor population is reflected in the total number of minicolumns which comprises the neocortex. In addition, a combination of migratory and proliferative disturbances have been found in the brains of autistic in the form of heterotopias and dysplasias – findings not unlike those produced in the Ang et al. (2006) murine model of prenatal ultrasound exposure (Bailey et al. 1998; Wegiel et al. 2010).

Increases in proliferation following ultrasound exposure can be seen in a number of cell types, such as fibroblasts, osteoblasts, chondrocytes, and monocytes (Doan et al. 1999; Zhang et al. 2003). Unfortunately, little research has been performed on broader cell types because ultrasound is used heavily as a therapeutic tool for bone repair, and therefore, there is less demand for applied research in other areas. Ultrasound effects on the mitogenic factor-releasing capacity of macrophages and their direct effects on proliferative rates of fibroblasts have however been studied, illustrating that indirect bioeffects from cell-to-cell interaction also follow ultrasound exposure (Young and Dyson 1990b). Given the effects ultrasound has on membrane permeability and the cellular growth which generally follows transient cell porosity, it will be interesting to note in future whether these results are replicated across cell types.

Some of the US-induced disturbances in proliferation are reflected in some of the autism genetics research performed to date. PTEN, for instance, is associated with a set of hamartomatous conditions with loss-of-function mutations; it is also significantly associated with autism and combined macrocephaly (McBride et al. 2010). PTEN lies upstream of pathways such as Akt and regulates the phosphorylation of $PIP_2$ to $PIP_3$; therefore, any loss of function of this protein leads to abnormal upregulation of Akt and changes in related pathways. MAPK/ERK is another aforemen-

tioned pathway which shares considerable crosstalk and co-regulation with the Akt pathway. *c-Met*, a proto-oncogene whose mutations are associated with nonsyndromic forms of autism, likewise lies upstream of MAPK/ERK and promotes its activity (Campbell et al. 2006). Its activation is also heavily inhibited by PTEN in a complex fashion, providing links through these various interactive pathways to the heterogeneous forms of autism (Abounader et al. 2004).

Ultrasound, like some of the syndromic and nonsyndromic mutations in autism, targets familiar pathways like MAPK/ERK, Akt, NFκB, Wnt, and numerous more (Hou et al. 2009; Olkku et al. 2010). These pathways are targets of pore-induced cellular signaling and activation of calcium-activated pathways, but many of these molecules have phosphorylation sites that may be directly oxidized through US exposure. As discussed earlier, Dishevelled, upon reduction, is downregulated irrespective of Wnt ligand activity, and thus, following ultrasonic oxidation, this pathway's activity may be upregulated. Oxidation can also inhibit HDACs and thereby upregulate canonical Wnt through a transcriptional means which can then be epigenetically inherited for multiple cellular generations. HDAC inhibition has previously been linked with autism, showing that prenatal valproic acid and/or carbamazepine exposure can increase risk of developing the condition (Rasalam et al. 2005). Both of these medications have a variety of secondary effects, but as one of their primary effects, they act as potent HDAC classes I and II inhibitors (Shimshoni et al. 2007). The MAPK/ERK pathways have also been found to regulate HDACs, linking their activity with chromatin remodeling and autism-associated mutations in some of the MAPK genes, such as MAPK3 (Zhou et al. 2000; Sanders et al. 2011). Ultrasound's effects on migration may also be echoed in the *RELN* gene which is associated with some nonsyndromic forms of autism. Reelin is an extracellular matrix protein which is intimately involved in neuronal migration, cell adhesion, and even synaptic plasticity, and any loss-of-function mutation inevitably causes extreme disruption of the cortex (Weeber et al. 2002). Other genes involved in cell adhesion, such as *CNTNAP2* whose loss of function is associated with autism, may provide phenotypic links to the loss of intercellular adhesion seen following US exposure (Pinto et al. 2010; Siegel et al. 1979).

Oxidative stress, like that following ultrasound exposure, has been associated with autism, although understanding in the field of study is still in its infancy. By-products of oxidative stress have been studied in autism at a number of levels, including the membrane level, in serum and urine, and at the level of specific endogenous and dietary antioxidants. For instance, increased levels of 8-hydroxy-2-deoxyguanosine and 8-isoprostane-F2α, both by-products of lipid peroxidation and thus indicators of the activity of this process, have been noted in the serum of autistic children as have lower levels of glutathione which is an important antioxidant and regulator of redox homeostasis (Ming et al. 2005; James et al. 2006). A poverty of production of the binding proteins, transferrin, an iron-binding protein, and ceruloplasmin, a copper-binding protein, has been noted in the conditions as well (Chauhan et al. 2004). Both iron and copper are potential catalysts of oxidation due to the ease with which they lose and gain electrons, and therefore, their binding proteins are important regulators of these catalysts' activities. Ultimately, lower levels of transferrin or ceruloplasmin

in the blood may suggest an increased risk to oxidative stress due to higher concentrations of free iron and copper. From an ecological standpoint, air pollution, particularly pollution due to heavy metals such as mercury, cadmium, and nickel, has also been associated with increased autism risk. These metals, similar to copper and iron, have the capacity to act as free radicals as well as to inactivate glutathione (Windham et al. 2006; Valko et al. 2006, for review). In summary, it is clear that redox homeostasis is disrupted in a portion of cases of autism, although by means unknown. Because redox dysregulation can be a sign of disrupted cellular health, it is difficult to postulate whether oxidative stress is a cause or a result of the conditions.

## 3 Discussion

While focusing on a few snippets of the larger ever-growing body of autism, research must seem like magnifying a molehill into a mountain and subsequently taking it out of context; instead we have tried to select examples which speak to an underlying theme, namely, one of dysregulation of cellular growth, migration, and differentiation in autism. And in that sense, heterogeneous autism may be a good example of "all roads lead to Rome" such that multiple avenues, including genetic mutation, epigenetic alteration, medication exposure, oxidative stress, diet, ultrasound, or a combination of effectors, may produce a common phenotype. As West-Eberhard (2005) states:

> [it] does not matter, for the form taken by the morphological change, whether the pivotal change . . . was induced by a mutation or by an environmental accident. The particular characteristics of the novel morphology . . . arose via mechanisms of developmental plasticity, not owing to the particular genetic or environmental change that may have induced them (p. 618).

Biological plasticity is reflected at numerous levels. In this chapter, we have focused on its plasticity at the molecular and cellular levels, illustrating that the activity of key pathways, reflected in the genetics of some cases of autism, can be altered through means other than DNA mutation and may instead be largely epigenetic. *Epigenetic* in this context refers to more than the alteration and inheritance of chromatin and methylation patterns but also to modes of inheritance that exist outside the cell, including that of its surrounding ecology. Mutations associated with syndromic and some nonsyndromic cases of autism, while still only comprising a minority of the larger spectrum, can help us pinpoint some of the particular molecular pathways which are involved in the broad spectrum of autism. It is this concept which we have earlier dubbed, "The Lowest Common Denominator of Autism" (Williams and Casanova 2010, 2011, for review). While genetics gives us vital information, it is obvious that autistic genotypes are heterogeneous, and so we must move to a higher level of comparison to identify trends, namely, the activity and timing of key molecular pathways involved in producing a common phenotype. To paraphrase West-Eberhard (2005) in this context, it does not matter *what* causes the alteration in activity of a given pathway but that it is altered *at all*. Given that

molecular pathways are not unlike a string of dominoes, it does not ultimately matter whether you target one domino or another within the grouping; the end result (genetic transcription or transcriptional suppression) is much the same. And the redundancy and cross-regulation of these various pathways likewise underlie the variability of biological plasticity. Therefore, if we apply this concept to prenatal ultrasound exposure, noting the commonalities its bioeffects share with both syndromic and nonsyndromic forms of autism, we can slowly begin to see how ultrasound may play some role in the conditions' etiology.

It has been extremely difficult to pin down ultrasound as a risk factor for autism. For one, funding and interest have been scarce because the idea of ultrasound as a teratogen to most people sounds like a pseudoscience, when in fact our understanding of its biophysical mechanics shows quite clearly that concern is justifiable. In reference to the modicum of work which has already been done on this topic, Grether et al. (2010) performed an epidemiological study which at face value provides evidence which would quell our concerns; however, as we learn more about the deregulation of the method and its variability of exposure in real medical practices, it becomes almost impossible to gauge risk by searching through medical records. In essence, we need well-controlled studies with well-defined parameters, and that includes animal studies which can give us even greater control within the laboratory and provide postmortem materials. Only in these ways can we clarify what role ultrasound plays in development and precisely how safety standards need to be tightened.

At one point in time, X-rays were used to excess in medical practice until eventually researchers realized that they were carcinogenic. This new tool was discovered in 1895 and was rapidly put to prolific and poorly regulated use. It was not until skin damage was linked with exposure that scientists and practitioners slowly began to improve safety regulations. By 1927, several decades later, there was an inkling of awareness of its mutagenic properties when Hermann Muller discovered that X-rays could promote genetic mutations in *Drosophila* (Muller 1927), and by the 1950s research suggested links between prenatal exposure and later development of cancer in childhood (e.g., see Giles et al. 1956). As discussed within the introduction of this chapter, the history of ultrasound shares parallels to X-ray in its early unregulated use, with a subsequent tightening of safety standards after the realization that ultrasound, while not mutagenic, can nevertheless cause tissue damage. Such is human nature to apply a novelty excessively until we discover its side effects.

Unfortunately, the last several decades have experienced a loosening of these regulations while very little of the growing body of research on ultrasound has been applied to its use in obstetrics. Very few scientists who study its effects on targeted drug therapy, on bone and cartilage regeneration, on gene delivery, and on intravascular dilation and imaging and numerous other studies for its application seem to publish any concerns regarding its use in fetal monitoring. In part, this is likely a reflection of the isolation in which each subfield generally resides. Developmental biologists are probably unfamiliar with the relevant literature, and scientists from other fields who do research ultrasound likely have little professional interest in publishing out of their disciplines. And for the developmental biologist, drudging up

literature on "ultrasound" and "safety" usually results in various old reviews citing the same outdated materials and reiterating the same pacifying statements, while in order to find the current science, the developmental biologist must search through foreign literature on gene transfection, delivery of medications to target organs utilizing ultrasound-enhanced microbubbles, or work elucidating bone repair by therapeutic ultrasound. A good deal of the science of ultrasound is known and available to the reader, but it is hidden by search engines and disciplinary isolation, and this will only be rectified once embryologists delve into these texts and apply them to their own field. And therefore it is our hope that this chapter will serve as a cross-disciplinary starting point for those scientists who wish to travel into less familiar territories.

## References

Abounader R, Reznik T, Colantuoni C, Martinez-Murillo F, Rosen EM, Laterra J (2004) Regulation of c-Met-dependent gene expression by PTEN. Oncogene 23:9173–9182

Adcock IM, Cosio B, Tsaprouni L, Barnes PJ, Ito K (2005) Redox regulation of histone deacetylases and glucocorticoid-mediated inhibition of the inflammatory response. Antioxid Redox Signal 7(1–2):144–152

Ang ES Jr, Gluncic V, Duque A, Schafter ME, Rakic P (2006) Prenatal exposure to ultrasound waves impacts neuronal migration in mice. Proc Natl Acad Sci U S A 103(34):12903–12910

Aylward EH, Minshew NJ, Field K, Sparks BF, Singh N (2002) Effects of age on brain volume and head circumference in autism. Neurology 59(2):175–183

Bailey A, Luthert P, Dean A, Harding B, Janota I, Montgomery M, Rutter M, Lantos P (1998) A clinicopathological study of autism. Brain 121(Pt 5):889–905

Banerjee D, Slack F (2002) Control of developmental timing by small temporal RNAs: a paradigm for RNA-mediated regulation of gene expression. Bioessays 24(2):119–129

Barnett SB, Ter Haar GR, Ziskin MC, Rott HD, Duck FA, Maeda K (2000) International recommendations and guidelines for the safe use of diagnostic ultrasound in medicine. Ultrasound Med Biol 26(3):355–366

Bennett MV, Zukin RS (2004) Electrical coupling and neuronal synchronization in the mammalian brain. Neuron 41(4):495–511

Berridge MJ, Lipp P, Bootman MD (2000) The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol 1(1):11–21

Buhimschi IA, Kramer WB, Buhimschi CS, Thompson LP, Weiner CP (2000) Reduction-oxidation (redox) state regulation of matrix metalloproteinase activity in human fetal membranes. Am J Obstet Gynecol 182(2):458–464

Campbell DL, Stamler JS, Strauss HC (1996) Redox modulation of L-type calcium channels in ferret ventricular myocytes. Dual mechanism regulation by nitric oxide and S-nitrosothiols. J Gen Physiol 108(4):277–293

Campbell DB, Sutcliffe JS, Ebert PJ, Militerni R, Bravaccio C, Trillo S et al (2006) A genetic variant that disrupts MET transcription is associated with autism. Proc Natl Acad Sci U S A 103(45):16834–16839

Campos LS, Leone DP, Relvas JB, Brakebusch C, Fässler R, Suter U, ffrench-Constant C (2004) β1 integrins activate a MAPK signalling pathway in neural stem cells that contributes to their maintenance. Development 131(14):3433–3444

Casanova MF (2004) White matter volume increase and minicolumns in autism. Ann Neurol 56:453

Casanova MF, Buxhoeveden DP, Switala AE, Roy E (2002) Minicolumnar pathology in autism. Neurology 58(3):428–432

Chauhan A, Chauhan V, Brown WT, Cohen I (2004) Oxidative stress in autism: increased lipid peroxidation and reduced serum levels of ceruloplasmin and transferrin– the antioxidant proteins. Life Sci 75(21):2539–2549

Cotterman R, Jin VX, Krig SR, Lemen JM, Wey A, Farnham PJ et al (2008) N-Myc regulates a widespread euchromatic program in the human genome partially independent of its role as a classical transcription factor. Cancer Res 68(23):9654–9662

Deng CX, Sieling F, Pan H, Cui J (2004) Ultrasound-induced cell membrane porosity. Ultrasound Med Biol 30(4):519–526

Dinno MA, Dyson M, Young SR, Mortimer AJ, Hart J, Crum LA (1989) The significance of membrane changes in the safe and effective use of therapeutic and diagnostic ultrasound. Phys Med Biol 34(11):1543–1552

Doan N, Reher P, Meghji S, Harris M (1999) In vitro effects of therapeutic ultrasound on cell proliferation, protein synthesis, and cytokine production by human fibroblasts, osteoblasts, and monocytes. J Oral Maxillofac Surg 57(4):409–419

Fiaschi T, Cozzi G, Raugei G, Formigli L, Ramponi G, Chiarugi P (2006) Redox regulation of beta-actin during integrin-mediated cell adhesion. J Biol Chem 281(32):22983–22991

Funato Y, Michiue T, Asashima M, Miki H (2006) The thioredoxin-related redox-regulating protein nucleoredoxin inhibits Wnt-beta-catenin signalling through dishevelled. Nat Cell Biol 8(5):501–508

Giles D, Hewitt D, Stewart A, Webb J (1956) Malignant disease in childhood and diagnostic radiation in utero. Lancet 1:447

Götz M, Stricker SH (2006) Go with the flow: signaling from the ventricle directs neuroblast migration. Nat Neurosci 9(4):470–472

Grether JK, Li SX, Yoshida CK, Croen LA (2010) Antenatal ultrasound and risk of autism spectrum disorders. J Autism Dev Disord 40(2):238–245

Griendling KK, Ushio-Fukai M (1998) Redox control of vascular smooth muscle proliferation. J Lab Clin Med 132(1):9–15

Groszer M, Erickson R, Scripture-Adams DD, Lesche R, Trumpp A, Zack JA et al (2001) Negative regulation of neural stem/progenitor cell proliferation by the *Pten* tumor suppressor gene in vivo. Science 294:2186–2189

Guirao B, Meunier A, Mortaud S, Aquilar A, Corsi JM, Strehl L et al (2010) Coupling between hydrodynamic forces and planar cell polarity orients mammalian motile cilia. Nat Cell Biol 12(4):341–350

Hatakeyama J, Bessho Y, Katoh K, Ookawara S, Fujioka M, Guillemot F et al (2004) *Hes* genes regulate size, shape and histogenesis of the nervous system by control of the timing of neural stem cell differentiation. Development 131:5539–5550

Ho SN (2006) Intracellular water homeostasis and the mammalian cellular osmotic stress response. J Cell Physiol 206(1):9–15

Hogg N, Kalyanaraman B (1999) Nitric oxide and lipid peroxidation. Biochim Biophys Acta 1411(2–3):378–384

Hormuzdi SG, Filippov MA, Mitropoulou G, Monyer H, Bruzzone R (2004) Electrical synapses: a dynamic signaling system that shapes the activity of neuronal networks. Biochim Biophys Acta 1662(1–2):113–137

Hou CH, Lin J, Huang SC, Hou SM, Tang CH (2009) Ultrasound stimulates NF-κB activation and iNOS expression via the Ras/Raf/MEK/ERK signaling pathway in cultured preosteoblasts. J Cell Physiol 220:196–220

Hsu HC, Fong YC, Chang CS, Hsu CJ, Hsu SF, Lin JG et al (2007) Ultrasound induces cyclooxygenase-2 expression through integrin, integrin-linked kinase, Akt, NF-κB and p300 pathway in human chondrocytes. Cell Signal 19(11):2317–2328

James SJ, Melnyk S, Jernigan S, Cleves MA, Halstead CH, Wong DH et al (2006) Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. Am J Med Genet 141B(8):947–956

Jana AK, Agarwal S, Chatterjee SN (1990) The induction of lipid peroxidation in liposomal membrane by ultrasound and the role of hydroxyl radicals. Radiat Res 124(1):7–14

Juffermans LJ, Dijkmans PA, Musters RJ, Visser CA, Kamp O (2006) Transient permeabilization of cell membranes by ultrasound-exposed microbubbles is related to formation of hydrogen peroxide. Am J Physiol 291:H1595–1601

Koshiyama K, Kodama T, Yano T, Fujikawa S (2006) Structural change in lipid bilayers and water penetration induced by shock waves: molecular dynamics simulations. Biophys J 91(6):198–205

Kumon RE, Aehle M, Sabens D, Parikh P, Kourennyi D, Deng CX (2007) Ultrasound-induced calcium oscillations and waves in Chinese hamster ovary cells in the presence of microbubbles. Biophys J 93(6):L29–L31

Lawrie A, Brisken AF, Franics SE, Wyllie D, Kiss-Toth E, Qwarnstrom EE et al (2003) Ultrasound-enhanced transgene expression in vascular cells is not dependent upon cavitation-induced free radicals. Ultrasound Med Biol 29(10):1453–1461

Lei SZ, Pan ZH, Aggarwal SK, Chen HS, Hartman J, Sucher NJ, Lipton SA (1992) Effect of nitric oxide production on the redox modulatory site of the NMDA receptor-channel complex. Neuron 8(6):1087–1099

Liebeskind D, Bases R, Koenigsberg M, Koss L, Raventos C (1981) Morphological changes in the surface characteristics of cultured cells after exposure to diagnostic ultrasound. Radiology 138:419–423

Mårtensson M, Olsson M, Segall B, Fraser AG, Winter R, Brodin L-A (2009) High incidence of defective ultrasound transducers in use in routine clinical practice. Eur J Echocardiogr 10:389–394

Mårtensson M, Olsson M, Brodin L-A (2010) Ultrasound transducer function: annual testing is not sufficient. Eur J Echocardiogr 11:801–805

McBride KL, Varga EA, Pastore MT, Prior TW, Manickam K, Atkin JF et al (2010) Confirmation study of PTEN mutations among individuals with autism or developmental delays/mental retardation and macrocephaly. Autism Res 3(3):137–141

McDonagh B, Tyther R, Sheehan D (2005) Carbonylation and glutathionylation of proteins in the blue mussel *Mytilus edulis* detected by proteomic analysis and Western blotting: actin as a target for oxidative stress. Aquat Toxicol 73(3):315–326

Medina MA, del Castillo-Olivares A, Schweigerer L (1992) Plasma membrane redox activity correlates with N-myc expression in neuroblastoma cells. FEBS Lett 311(2):99–101

Ming X, Stein TP, Brimacombe M, Johnson WG, Lambert GH, Wagner GC (2005) Increased secretion of a lipid peroxidation biomarker in autism. Prostaglandins Leukot Essent Fatty Acids 73(5):379–384

Minkel JR (2010) Focus: how to open a hole in a cell. Phys Rev Focus 26:2

Muller HJ (1927) Artificial transmutation of the gene. Science 66(1699):84–87

Murata H, Ihara Y, Nakamura H, Yodoi J, Sumikawa K, Kondo T (2003) Glutaredoxin exerts an antiapoptotic effect by regulating the redox state of Akt. J Biol Chem 278(50):50226–50233

Olkku A, Leskinen JJ, Lammi MJ, Hynynen K, Mahonen A (2010) Ultrasound-induced activation of Wnt signaling in human MG-63 osteoblastic cells. Bone 47(2):320–330

Pébay A, Peshavariya H, Wong RCB, Dusting GJ (2011) Non-classical signalling mechanisms in stem cells. In: Atwood CS (ed) Embryonic stem cells: the hormonal regulation of pluripotency and embryogenesis. InTech, Rijeka, pp 317–336

Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R et al (2010) Functional impact of global rare copy number variation in autism spectrum disorders. Nature 466(7304):368–372

Pohl P, Antonenko YN, Rosenfeld E (1993) Effect of ultrasound on the pH profiles in the unstirred layers near planar bilayer lipid membranes measured by microelectrodes. Biochim Biophys Acta 1152:155–160

Rahman I, Marwick J, Kirkham P (2004) Redox modulation of chromatin remodeling: impact on histone acetylation and deacetylation, NF-κB and pro-inflammatory gene expression. Biochem Pharmacol 68:1255–1267

Ranjan P, Anathy V, Burch PM, Weirather K, Lambeth JD, Heintz NH (2006) Redox-dependent expression of cyclin D1 and cell proliferation by Nox1 in mouse lung epithelia cells. Antioxid Redox Signal 8(9–10):1447–1459

Rasalam AD, Hailey H, Williams JH, Moore SJ, Turnpenny PD, Lloyd DJ et al (2005) Characteristics of fetal anticonvulsant syndrome associated autistic disorder. Dev Med Child Neurol 47(8):551–555

Reddy A, Caler EV, Andrews NW (2001) Plasma membrane repair is mediated by Ca(2+)-regulated exocytosis of lysosomes. Cell 106(2):157–169

Reher P, Doan N, Bradnock B, Meghji S, & Harris M (1999) Effect of ultrasound on the production of IL-8, basic FGF and VEGF. Cytokine 11:416–423

Reher P, Harris M, Whiteman M, Hai HK, Meghji S (2002) Ultrasound stimulates nitric oxide and prostaglandin $E_2$ production by human osteoblasts. Bone 31:236–241

Riesz P, Kondo T (1992) Free radical formation induced by ultrasound and its biological implications. Free Radic Biol Med 13:247–270

Roy S, Khanna S, Sen CK (2008) Redox regulation of the VEGF signaling pathway and tissue vascularization: hydrogen peroxide, the common link between physical exercise and cutaneous wound healing. Free Radic Biol Med 44(2):180–192

Sanders SJ, Ercan-Sencicek AG, Hus V, Luo R, Murtha MT, Moreno-De-Luca D et al (2011) Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. Neuron 70(5):863–885

Sarbassov DD, Sabatini DM (2005) Redox regulation of the nutrient-sensitive raptor-mTOR pathway and complex. J Biol Chem 280(47):39505–39509

Schneider-Kolsky ME, Ayobi Z, Lombardo P, Brown D, Kedang B, Gibbs ME (2009) Ultrasound exposure of the foetal chick brain: effects on learning and memory. Int J Dev Neurosci 27:677–683

Sheiner E, Shoham-Vardi I, Abramowicz JS (2007) What do clinical end users know regarding safety of ultrasound during pregnancy? J Ultrasound Med 26:319–325

Shen Q, Goderie SK, Jin L, Karanth N, Sun Y, Abramova N et al (2004) Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. Science 304(5675):1338–1340

Shimshoni JA, Dalton EC, Jenkins A, Eyal S, Ewan K, Williams RS et al (2007) The effects of central nervous system-active valproic acid constitutional isomers, cyclopropyl analogs, and amide derivatives on neuronal growth cone behavior. Mol Pharmacol 71(3):884–892

Siegel E, Goddard J, James AE Jr, Siegel EP (1979) Cellular attachment as a sensitive indicator of the effects of diagnostic ultrasound exposure on cultured human cells. Radiology 133: 175–179

Tervonen TA, Louivuori V, Sun X, Hokkanen ME, Kratochwil CF, Zebryk P, et al. (2009). Abberant differentiation of glutamatergic cells in neocortex of mouse model for fragile X syndrome. Neuro of Dis 33:250–259

Tyler WJ, Tufail Y, Finsterwald M, Tauchmann ML, Olson EJ, Majestic C (2008) Remote excitation of neuronal circuits using low-intensity, low-frequency ultrasound. PLoS One 3(10):e3511

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160(1):1–40

Way SW, McKenna J, Mietzsch U, Reith RM, Wu HCJ & Gambello MJ (2009) Loss of Tsc2 in radial glia models the brain pathology of tuberous sclerosis complex in the mouse. Hum Mol Genet 18:1252–1265

Weeber EJ, Beffert U, Jones C, Christian JM, Forster E, Sweatt JD et al (2002) Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. J Biol Chem 277(42):39944–39952

Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Marchi E et al (2010) The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. Acta Neuropathol 119(6):755–770

West-Eberhard MJ (2005) Phenotypic accommodation: adaptive innovation due to developmental plasticity. J Exp Zool B Mol Dev Evol 304(6):618

Williams EL, Casanova MF (2010) Autism or autisms? Finding the lowest common denominator. Bol Asoc Med P R 102(4):17–24

Williams EL, Casanova MF (2011) Above genetics: lessons from cerebral development in autism. Transl Neurosci 2(2):106–120

Wiltse J (2005) Mode of action: Inhibition of histone deacetylase, altering WNTdependent gene expression, and regulation of beta-catenin-developmental effects of valproic acid. CRC Critical Rev in Toxicol 35:727–738

Windham GC, Zhang L, Gunier R, Croen LA, Grether JK (2006) Autism spectrum disorders in relation to distribution of hazardous air pollutants in the San Francisco Bay Area. Environ Health Perspect 114(9):1438–1444

Yamamoto K, Takahashi T, Asahara T, Ohura N, Sokabe T, Kamiya A et al (2003) Proliferation, differentiation, and tube formation by endothelial progenitor cells in response to shear stress. J Appl Physiol 95(5):2081–2088

Yang F, Gu N, Chen D, Xi X, Zhang D, Li Y et al (2008) Experimental study on cell self-sealing during sonoporation. J Control Release 131(3):205–210

Young SR, Dyson M (1990a) The effect of therapeutic ultrasound on angiogenesis. Ultrasound Med Biol 16(3):261–269

Young SR, Dyson M (1990b) Macrophage responsiveness to therapeutic ultrasound. Ultrasound Med Biol 16(8):809–816

Zhang ZJ, Huckle J, Francomano CA, Spencer RG (2003) The effects of pulsed low-intensity ultrasound on chondrocyte viability, proliferation, gene expression and matrix production. Ultrasound Med Biol 29(11):1645–1651

Zhang X, Odom DT, Koo S-H, Conkright MD, Canettieri G, Best J et al (2005) Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc Natl Acad Sci U S A 102(12):4459–4464

Zhou X, Richon VM, Wang AH, Yang XJ, Rifkind RA, Marks PA (2000) Histone deacetylase 4 associates with extracellular signal-regulated kinases 1 and 2, and its cellular localization is regulated by oncogenic Ras. Proc Natl Acad Sci U S A 97(26):14329–14333

Zhu D, Tan KS, Zhang X, Sun AY, Sun GY, Lee JC (2005) Hydrogen peroxide alters membrane and cytoskeleton properties and increases intercellular connections in astrocytes. J Cell Sci 118(Pt 16):3695–3703