GENETIC BASIS OF MECHANOSENSORY TRAITS IN HUMANS

Dissertation

zur Erlangung des akademischen Grades

Doctor rerum naturalium

(Dr. rer. nat.)

Vorgelegt von

Henning Frenzel

Angefertigt in der Arbeitsgruppe „Molecular Physiology of Somatic Sensation“ des Max-Delbrück-Centrums für Molekulare Medizin unter der Leitung von Prof. Dr. Gary Lewin

Eingereicht im Fachbereich Biologie, Chemie, Pharmazie
der Freien Universität Berlin

Berlin, November 2010

1 Gutachter: **Prof. Dr. F.G. Rathjen**
MDC Berlin

2. Gutachter: **Prof. Dr. G. R. Lewin**
MDC Berlin**.**

Disputationstermin: 06.01.2011

Erklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne unerlaubte Hilfe durchgeführt habe.

Berlin, den

Danksagung

Ich möchte Gary Lewin dafür danken, dass er mir die Möglichkeit gab, an diesem Projekt zu arbeiten und mich bei der Durchführung immer unterstützte und motivierte.
Manfred Gross danke ich für die hervorragende Zusammenarbeit mit der Klinik für Audiologie und Phoniatrie.
Ich bedanke mich bei Jörg Bohlender für die Koordination der Studien in der Klinik für Audiologie und Phoniatrie, sowie für die gute Zusammenarbeit, vor allem bei der Untersuchung von verschiedenen Patientengruppen.
Bei Katrin Pinsker bedanke ich mich für die Koordination und Durchführung der Untersuchungen in der Klinik für Audiologie und Phoniatrie.
Ich bedanke mich bei Stefan Lechner für die gute Zusammenarbeit bei dem „molekularen“ Teil meiner Arbeit aber auch für die zahlreichen Diskussionen über die anderen Studien.
Bei Susanna Dietz und Christina Basler möchte ich mich für die Durchführung der Audiometrie bedanken.
Bei Bärbel Wohlleben und José Millan bedanke ich mich für die Hilfe bei der Rekrutierung von Testpersonen.
Jens Tank danke ich dafür, dass er mir die Funktion und Messung des Baroreflex erklärte.
Ich bedanke mich bei Norbert Hübner dafür, dass er die Durchführung der Zygotieanalysen und die Microarray-Analysen ermöglichte.
Außerdem bedanke ich mich bei allen anderen Mitgliedern in der AG Lewin, der Klinik für Audiologie und Phoniatrie und der (ehemaligen) AG Jordan, die mich unterstützt haben.

Mein besonderer Dank gilt den zahlreichen Studienteilnehmern, ohne die diese Arbeit nicht möglich gewesen wäre.

TABLE OF CONTENTS

1. Summary / Zusammenfassung

1.1 Summary

The aim of this study was to explore the genetics of sensory traits. In a classical twin study two aspects of touch sensitivity were investigated, tactile acuity and vibration sensitivity. We could demonstrate for the first time that there is a heritable component to touch sensitivity in humans. This shows the suitability of quantitative sensory testing for genome-wide approaches to study the genetics of touch sensitivity. Genetic components could also be shown for measures of hearing, the vascular baroreflex and cutaneous temperature sensitivity.

We further hypothesized that there are common genetic factors, which are involved in the different mechanosensory systems of the body. By assessing different sensory systems in healthy individuals we could show that there is cross-correlation between touch sensitivity, the performance of the auditory system and the function of the vascular baroreflex on a phenotypic level.

To learn more specifically about a possible involvement of hearing genes in touch sensitivity, we determined the tactile acuity and vibration sensitivity in different cohorts affected by either nonsyndromic congenital hearing impairment or the Usher syndrome, the most common form of genetic deaf-blindness. We found reduced overall touch sensitivity in the cohort of nonsyndromic congenital deaf / hearing impaired people, reduced vibration sensitivity in Usher type 1 patients carrying mutations in the gene *Myosin7a*, and reduced tactile acuity in Usher type 2 patients with mutations in the *Ush2a* gene. A cohort of Usher type 1 patients with defective *Cadherin23* genes and a cohort of Usher type 2 patients with unidentified mutations were not affected. This is the first report concerning impaired touch sensitivity being associated with congenital hearing impairment. The association does not affect all types of congenital hearing impairment in the same way, suggesting that some genes are involved in both touch and hearing and that not a central effect, caused by missing auditory input, is primarily responsible. Baroreflex function was also measured in Usher type 2 patients, but no difference compared to a control cohort was detected.

To assess the effects of tactile training, touch sensitivity was tested in a cohort of blind people and tactile acuity was found to be enhanced whereas vibration sensitivity was not.
In a second project, a screen for genes that are involved in cutaneous mechanotransduction was performed by determining gene expression in the developing mouse embryo and matching expression profiles to the emergence of mechanosensitivity of sensory neurons during development. We evaluated known candidate genes and in a genome-wide approach, using expression microarrays, we identified a set of approximately 50 genes that can be considered as new candidates for being involved in mechanotransduction.

1.2 Zusammenfassung

In dieser Studie sollte die Genetik von verschiedenen sensorischen Systemen des Körpers untersucht werden. In einer klassischen Zwillingsstudie wurden zwei Aspekte der Tastempfindlichkeit untersucht, die Tastgenauigkeit und die Vibrationsempfindlichkeit. Wir konnten zum ersten mal einen erblichen Anteil an der Tastempfindlichkeit beim Menschen nachweisen. Dies zeigt, das quantitative sensorische Tests dafür geignet sind, in genomweiten Ansätzen die Genetik des Tastsinns zu untersuchen. Außerdem konnten genetische Komponenten beim Hören, dem vaskulären Baroreflex und der Temperaturempfindlichkeit der Haut gezeigt werden.
Wir stellten die Hypothese auf, daß es gemeinsame genetische Faktoren in den verschiedenen mechanosensorischen Sytemen des Körpers gibt. Indem wir die verschiedenen sensorischen Systeme in gesunden Individuen verglichen haben, konnten wir zeigen, das es auf der phänotypischen Ebene Korrelationen zwischen dem Tastsinn, dem Hören und dem vaskulären Baroreflex gibt.
Um mehr über einen möglichen Zusammenhang zwischen Genen zu erfahren, die für den Hörsinn verantwortlich sind und der Tastempfindlichkeit, haben wir die Tastgenauigkeit und die Vibrationsempfindlichkeit in verschiedenen Kohorten, die entweder von nichtsyndromaler angeborener Gehörlosigkeit / Schwerhörigkeit betroffen sind oder vom Usher Syndrom, der häufigsten Form genetisch bedingter Taub-Blindheit. Wir fanden die Tastempfinfdlichkeit in beiden Tests bei der Kohorte der nichtsyndromal

hörgeschädigten reduziert, bei Usher Typ 1 Patienten, die Mutationen im Gen *Myosin7a* tragen, die Vibrationsempfindlichkeit reduziert und bei Usher Typ 2 Patienten mit defekten *Ush2a* Genen die Tastgenauigkeit reduziert. Eine Kohorte mit Usher Typ 1 Patienten mit Mutationen im Gen *Cadherin23* und eine Kohorte mit Usher Typ 2 Patienten, bei denen die Mutation nicht bekant war, waren nicht betroffen. Dies ist der erste Bericht über eine eingeschränkte Tastfähigkeit in Zusammenhang mit angeborener Hörstörung. Ein Zusammenhang besteht aber nicht bei allen untersuchten Formen der angeborenen Hörstörung, was nahelegt, daß einige Gene sowohl am Hören als auch am Tastsinn beteiligt sind und daß es sich nicht um einen zentralen Effekt handelt, der durch das Fehlen von auditorischen Reizen bedingt ist. Die Funktion des Baroreflexes wurde bei Usher Typ 2 Patienten untersucht, aber keine Veränderung festgestellt.

Um dei Auswirkung von taktilem Training zu untersuchen, wurde die Tastempfindlichkeit in einer Kohorte Blinder untersucht. Hierbei zeigte sich die Tastgenauigkeit erhöht, wohingegen die Vibrationsempfindlichkeit unverändert war.

In einem zweiten Projekt wurde nach Genen gesucht, die an der Mechanotransduktion beteiligt sind, indem die Expression von Genen im sich entwickelndem Maus-Embryo bestimmt und mit dem Auftreten der mechanischen Erregbarkeit von sensorischen Neuronen während der Entwicklung verglichen wurde. So konnten wir bekannte Kandidatengene bewerten und in einem genomweiten Ansatz ungefähr 50 Gene identifizieren, die als Kandidaten für eine Rolle in der Mechanotransduktion angesehen werden können.

2 Introduction

In the ever changing environment in which we move it is vital to continuously obtain information about our surroundings. At the same time we rely on information about the status of our own body. To accomplish this, our sensory systems have to detect and perceive stimuli of diverse physical nature. The detection of different stimuli is accomplished by specialized receptor structures of the nervous system or by structures of non-neural origin that subsequently transmit the information to the nervous system. Different receptor types in humans are able to detect light, chemical, thermal and mechanical stimuli, whereas other organisms are also able to detect electric and magnetic fields. Whereas some properties of the stimulus, such as intensity or change in intensity, are already coded by receptors themselves, other properties, such as direction or stimulus origin, are obtained by further processing in the nervous system. The direct input from our sensory systems occupies a large portion of our experience of consciousness. On the other hand, we are aware of only a fraction of the information that is processed by our sensory systems. There are also sensory systems that we never experience directly such as input from sensory systems that detect changes in blood pressure.

Whereas light, odors and flavors are detected exclusively by specialized organs, mechanical stimuli are detected by a variety of different sensory systems. Mechanical stimuli are detected by different cutaneous mechanoreceptors, which are spread throughout the body surface, similar to thermoreceptors. These mechanoreceptors are required for sensation of both touch and mechanical pain. There are additional mechanoreceptors deep inside the body that function as proprioceptors, which detect the positions of the body parts by acting as stretch detectors or tension detectors. Furthermore, transduction of mechanical stimuli into electrical signals is also the mechanism that underlies the function of the ear. In the cochlea, the incoming sound waves elicit the mechanical stimulation of the hair cell stereocilia, which represent different sound frequencies according to their position along the cochlea. In the vestibular system of the inner ear, rotation around the three axes, as well as linear movements, lead to the mechanical stimulation of receptors and generate our sense of balance and movement.

The molecular mechanism of transduction at the cellular level is well known for most sensory systems. In the light sensitive receptor cells of the mammalian eye, the rods and cones, the interaction of the incoming light with Rhodopsin molecules leads to the activation of a G-protein called Transducin. The active α-subunit of Transducin activates a phosphodiesterase that breaks down cyclic GMP to GMP. The reduction of cyclic GMP levels leads to closure of cyclic nucleotide gated ion channels and thus to a reduction of the actual transduction current, which ultimately leads to reduction of glutamate release from the receptor cells. Reduction of glutamate release is the signal to the neuronal cells that is generated by the receptor cells in response to exposure to light (Sung and Chuang 2010). Most smell and taste receptors also function on the basis of G-protein coupled receptor activation, which in this case leads to a release of neurotransmitters (Roper 2007; Kaupp 2010). In contrast to phototransduction and the chemical senses the molecular mechanisms of mechanotransduction are not well understood. In somatic mechanotransduction a reason for this is that this sensory system is dispersed throughout the body, which makes experimental approaches difficult. There are also no known nonsyndromic diseases where the primary symptom is a dysfunction of the somatic mechanoreceptors as it is the case for hearing where many genes are known that cause nonsyndromic deafness. However, the structure of the actual transduction complex in the ear is only partially described. It is also unknown if there are molecular mechanisms that are shared in the different mechanotransduction processes throughout the body.

2.1 The cutaneous sensory system

The peripheral sensory system of vertebrate skin is able to detect different physical stimuli. Touch sensation is mediated by low-threshold mechanoreceptors. There are also thermoreceptors that sense cold and warmth, as well as nociceptors, which detect painful stimuli that can be of thermal, mechanical or chemical nature. Transduction of these different stimuli is accomplished by the endings of sensory nerves. The cell bodies of these nerves are located in the dorsal root ganglia (DRG) (**Figure 1**). The axon branches and one branch innervates the receptive fields in the skin, whereas the other enters the spinal cord. Below, the different types of cutaneous sensory receptors are described. It is

important to note that sensory receptors that are deep inside the body, such as proprioreceptors, that monitor the position of the limbs or visceral nociceptors, also originate from DRG neurons (Wood 2004; Macefield 2005).

It is also important to note that the skin of the face and parts of the head are innervated, not by DRG neurons, but by neurons that emerge from the and cranial ganglia (Purves 2001).

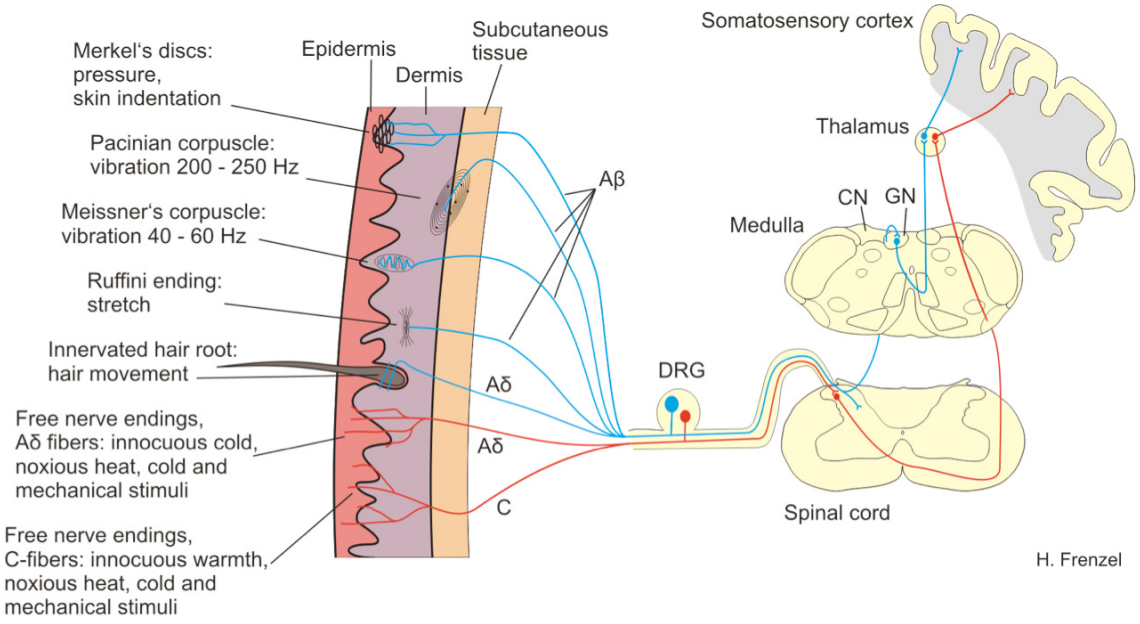

Figure 1: Schematic representation of the cutaneous sensory system. See text for description. Blue lines depict low threshold mechanosensory fibers and respective ascending fibers, red lines depict thermal- and nociceptors and respective ascending fibers. Aβ, Aδ and C stands for the respective fiber type; DRG = dorsal root ganglion; GN = gracile nucleus; CN = cuneate nucleus.

2.2 Mechanoreceptors

Most sensory afferent fibers that mediate touch sensation are large diameter and have thick myelin sheets, which give them a high conduction velocity of 35-70 m/s in humans (Johnson, Yoshioka et al. 2000), which are called Aβ-fibers. The axons of mechanoreceptor fibers terminate in five different types of non-neuronal structures in the skin. Fibers that terminate in the Merkel's discs, an accumulation of Merkel cells at the border between the epidermis and dermis, are sensitive to pressure applied to, and indentations of, the skin (Werner and Mountcastle 1965; Knibestol and Vallbo 1980; Phillips and Johnson 1981). Neurons that terminate in the subcutaneous Ruffini corpuscles are sensitive to skin stretch (Edin 1992). Merkel's discs and Ruffini corpuscles are both slowly adapting (SA), and respond during a static stimulus application. The corresponding fibers are called SAI (slowly adapting) and SAII fibers, respectively (Vallbo, Olausson et al. 1995). The other type of low threshold mechanoreceptors are the rapidly adapting (RA) fibers. RAI fibers innervate subcuteaneous Pacinian corpuscles and are responsive to high frequency vibrations with a maximum sensitivity between 200 and 250 Hz (Sato 1961; Mountcastle, LaMotte et al. 1972). RAII fibers innervate Meissner's corpuscles that are located in the ridges of the dermis protruding into the epidermis. RAII fibers are sensitive to low frequency vibrations with a maximum sensitivity from 40 to 60 Hz (Mountcastle, LaMotte et al. 1972) and may be involved in grip control (Macefield, Hager-Ross et al. 1996). An exception to the low threshold mechanoreceptors are the highly sensitive fibers that innervate hair roots and respond to hair displacement (Lewin and Moshourab 2004), as they are innervated by Aδ, not Aβ, fibers, that are smaller in diameter and have a lower conduction velocity between 2 and 30 m/s (Adriaensen, Gybels et al. 1983).

The transduction of the mechanical stimulus is thought to be accomplished in the endings of the sensory neurons and not in the non-neuronal structures that they innervate. This was shown by receptors still responding to mechanical stimulation, after the removal of all non-neuronal tissue. This mechanical responsiveness has been shown for fibers innervating both Pacinian corpuscles and Merkel cells (Loewenstein and Rathkamp 1958; Mills and Diamond 1995). Another argument for the mechanosensitivity of the neurons

themselves is their characteristics when isolated. Cultured DRG neurons fire action potentials in response to mechanical stimulation and potential transduction currents with varying adaption kinetics can be recorded (McCarter, Reichling et al. 1999; Drew, Rohrer et al. 2004; Hu and Lewin 2006; Lechner, Frenzel et al. 2009), which is a rapidly adapting current in mechanoreceptor neurons.

As mentioned before, the molecular nature and structure of the complex that is responsible for transducing mechanical stimuli is not well understood. Mechanotransduction is better understood in the nematode worm *Caenorhabdtis elegans* (Bounoutas and Chalfie 2007) and there is evidence that homologues of the genes that are involved in mechanotransduction in *C. elegans* are also involved in vertebrate mechanotransduction. When the *amiloride sensitive cation channel 2* (*Asic2*) gene was deleted in a knock-out mouse model (Price, Lewin et al. 2000), RA fibers showed a reduced sensitivity, whereas in *Asic3*$^{-/-}$ mice, the sensitivity of RA fibers was enhanced (Price, McIlwrath et al. 2001). However, the proposed mechanotransduction currents in cultured DRG neurons from *Asic2*$^{-/-}$, *Asic3*$^{-/-}$ double knock-out mice were normal (Drew, Rohrer et al. 2004; Lechner, Frenzel et al. 2009). Another homologue of a *C. elegans* gene (MEC-2) involved in mechanotransduction is *Slp3*. *Slp3*$^{-/-}$ mice showed a reduced performance in a tactile discrimination task. Furthermore, it was shown that in *Slp3*$^{-/-}$ mice, one third of the mechanoreceptor neurons were insensitive to mechanical stimulation. Reduced numbers of mechanosensitive neurons could be shown in extracellular recordings from cutaneous sensory fibers, as well as in recordings of mechanosensitive currents in cultured DRG neurons (Wetzel, Hu et al. 2007). Involvement of genes homologous to mechanotransduction genes in *C. elegans* argues for a gating spring model of mechanotransduction which has been hypothesized for *C. elegans* (Bounoutas and Chalfie 2007). In the gating spring model, the transduction channel is thought to be connected to the cytoskeleton and to the extracellular matrix by tether proteins instead of a direct opening of channels by membrane stretch. Recent results from a study in mice are also in favor of a gating spring model as an extracellular tether has been identified that appears to be necessary for neurons to be mechanosensitive (Hu, Chiang et al. 2010).

The central axonal branch of the mechanoreceptor neurons emerging from the DRG enters the spinal cord at the dorsal horn (**Figure 1**). Whereas collaterals of these DRG neurons project onto neurons in lamina III – V, the fibers ascend to the medulla, where they project onto interneurons in the gracile nucleus and the cuneate nucleus. The interneurons in the medulla cross to the contralateral side and then ascend to the thalamus. From here the signals are relayed to the somatosensory cortex (Purves 2001).

2.3 Cold and warmth detection

The fibers that mediate cooling and warming sensations do not terminate in specialized structures, but branch in the skin as free nerve endings. The fibers that mediate cooling sensation in the skin are Aδ fibers (Darian-Smith, Johnson et al. 1973; Dubner, Sumino et al. 1975; Kenshalo and Duclaux 1977). These Aδ cold sensitive fibers fire action potentials at a steady, low rate between 20 and 40 °C and when the temperature decreases within this range the firing rate goes up proportionally to the experienced temperature decrease and adapts afterwards.

Warming of the skin leads to responses from a population of small unmyelinated fibers, called C-fibers, which have conduction velocities under 2 m/s and that also branch as free nerve endings in the skin. They fire at a steady rate at temperatures between 30 and 50 °C and the firing rate increases upon warming of the skin proportionally to the temperature step (Darian-Smith, Johnson et al. 1979; Duclaux and Kenshalo 1980).

Various transient receptor potential (TRP) ion channels have been shown to be expressed in sensory neurons and some of them are temperature gated (Dhaka, Viswanath et al. 2006) and thus are good candidates to transduce temperature stimuli. Indeed a role for TRPM8 in cold sensation could be demonstrated by showing that in *Trpm8*$^{-/-}$ mice, sensitivity to cold stimuli has been strongly reduced (Bautista, Siemens et al. 2007; Dhaka, Murray et al. 2007). A role for TRPV3 in the behavioral response to warmth stimuli has been shown (Moqrich, Hwang et al. 2005), but no evidence on a physiological level has been reported.

The axons of nerves that mediate temperature sensation also enter the spinal cord in the dorsal horn, but terminate in superficial laminae I and II. Here the signals are relayed to

2nd order neurons which cross to the contralateral ventral side of the spinal cord where they ascend to the thalamus. In the thalamus the signals are relayed to the primary somatosensory cortex. This ascending pathway is called the anterolateral system (Purves 2001).

2.4 Nociceptors

Thermal and mechanical stimuli that are perceived as painful are detected by different fibers than when the respective stimuli are innocuous. Nociceptive fibers branch in the skin as free nerve endings, similar to the warmth and cold receptors. Aδ fibers respond to noxious mechanical stimuli (Burgess and Perl 1967), but can also respond to noxious temperatures of more than 40 °C (Beck, Handwerker et al. 1974; Georgopoulos 1976) and are thought to mediate the first, sharp pain that gives information about the intensity and location of a stimulus (Adriaensen, Gybels et al. 1983). The second, more diffuse pain felt subsequently is mediated by C-fibers. Nociceptive C-fibers can be activated by noxious mechanical stimuli (Iggo 1960) and / or by noxious heat (Bessou and Perl 1969; Torebjork and Hallin 1974).

Cold stimuli perceived as painful (below 20 °C) are detected by Aδ fibers (Georgopoulos 1976; Simone and Kajander 1997), some of which are also mechanonociceptors. There are also some C-fibers that are mechano- and cold sensitive (Bessou and Perl 1969; Torebjork and Hallin 1974; Georgopoulos 1976).

The molecular nature of the transducing structures involved in nociception remains largely unknown. Again, TRP ion channels have been proposed to play a role in thermal nociception because of their biophysical properties, but knock-out mouse models for TRPA1 (Bautista, Jordt et al. 2006), which is activated at temperatures below 17 °C, as well as for TRPV1 (Davis, Gray et al. 2000), which is activated at temperatures above 42 °C, failed to show obvious defects in thermal related behavior and normal response properties of single sensory fibers persist (Woodbury, Zwick et al. 2004; Kwan, Glazer et al. 2009).

The ascending pathway of the nociceptive signals to the somatosensory cortex is the anterolateral system, as described for the temperature detection above.

2.5 Hearing

The ear is able to detect sound waves at frequencies between 20 Hz and 20 kHz over an intensity range that spans six orders of magnitude. The sound waves strike the ear drum (**Figure 2**) and are amplified when they are transmitted to the relatively small oval window and into the cochlea. The sound waves, now transmitted to the perilymph, travel through the scala tympani and through the scala vestibuli. Between the scala tympani and the scala vestibuli lies the organ of corti which contains the hair cells, the receptor cells, that transduce the mechanical stimulus. The mechanical characteristics of the organ of corti differ along the span of the cochlea with the result that different sections vibrate in response to specific sound frequencies (Muller 1991). This leads to the mechanical stimulation of the hair cells that lie between the basilar and tectorial membrane, generating a receptor current (Corey and Hudspeth 1983; Crawford, Evans et al. 1989). The epithelial hair cells each have one bundle of sterocilia that connects to the tectorial membrane. The site of mechanotransduction is at the top of the stereocilia (Beurg, Fettiplace et al. 2009). The top of the stereocilia are connected to the adjacent larger sterocilia by tip links (Pickles, Comis et al. 1984), these tip links are proposed to be tethers connected to the mechanically gated transduction channel analogous to the gating spring mechanotransduction complex proposed for *C. elegans* (Bounoutas and Chalfie 2007). In the organ of corti there is one row of inner hair cells and three rows of outer hair cells. The actual electrical signal is generated by the inner hair cells and relayed by synaptic transmission to the auditory nerve (Hudspeth and Corey 1977).

Sensitivity and frequency selectivity is enhanced by actively amplifying the waves travelling along the cochlea, that can have an amplitude of as low as 10^{-11} m (Sellick, Patuzzi et al. 1982; Dallos 1992), and the outer hair cells are thought to accomplish this. Two mechanisms could play a role in this process, hair bundle motility (Brownell, Bader et al. 1985; Zheng, Shen et al. 2000) and the ability of the lateral membrane of the outer hair cells to change its size and thus change the length of the cells (Crawford and Fettiplace 1985; Fettiplace 2006). The otoacoustic emissions that are emitted from the inner ear (Kemp 1978), are interpreted as a result of this amplification process. These otoacoustic emissions are also used for clinical evaluation of cochlear function.

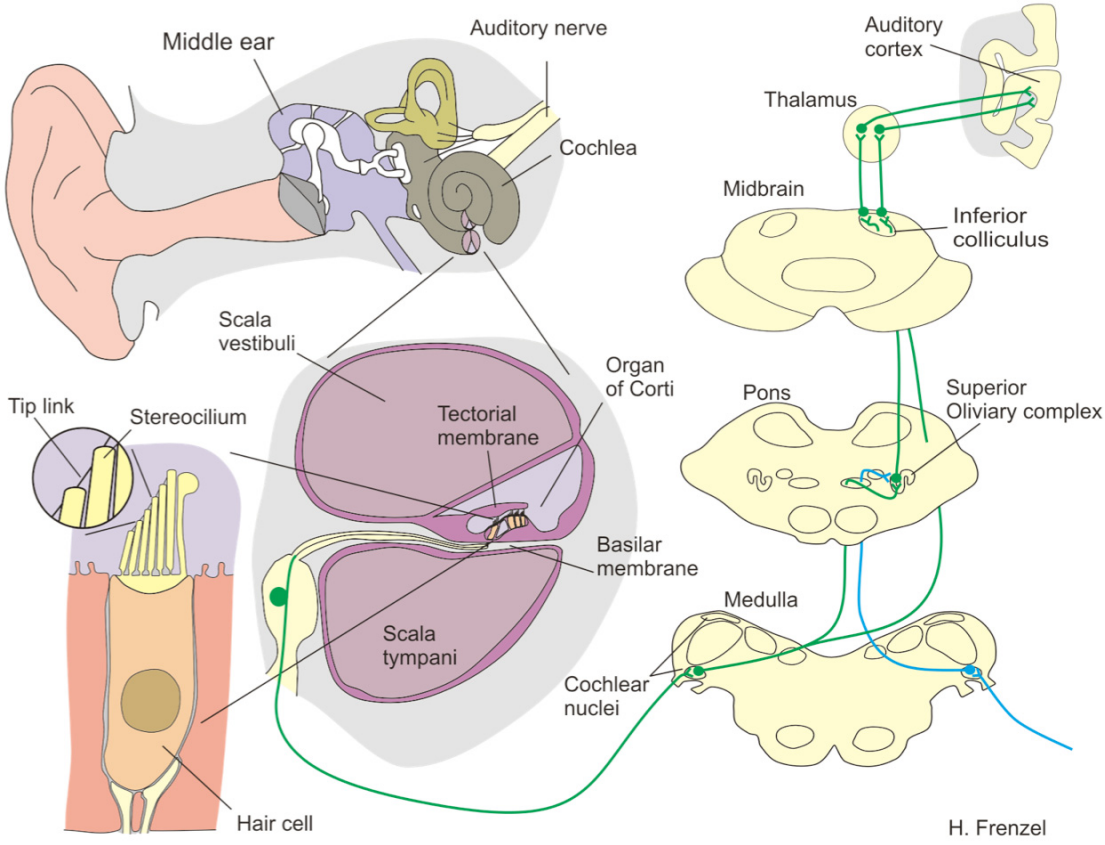

Figure 2: Schematic representation of the auditory system. See text for description. Green lines depict fibers that originate in the right ear and the respective ascending fibers; blue lines depict fibers that originate in the left ear.

The molecular identity of the transduction ion channel is not known and no clear candidates have emerged so far. Protocadherin15 and Cadherin23 have been shown to be the molecules that constitute the tip link (Siemens, Kazmierczak et al. 2002; Ahmed, Goodyear et al. 2006; Kazmierczak, Sakaguchi et al. 2007) and can be regarded as part of the transduction complex. The two motor proteins Myosin1C and Myosin7a have been shown to play a role in adaptation of the transduction current, as seen in studies in Myosin1C and Myosin7a mutant mice, where adaptiaton is impaired (Kros, Marcotti et al. 2002; Stauffer, Scarborough et al. 2005) and thus Myosin1C and Myosin7a can also be viewed as a part of the transduction complex.

The auditory nerve projects to the cochlear nucleus in the medulla and from there the signals ascend in parallel pathways. The integration of signals from both ears to extract information about the spatial origin of the auditory stimulus occurs in the superior

oliviary complex in the pons. The signals from one ear are processed with the information of the other ear and converge again in the contralateral inferior colliculus of the midbrain and are relayed in the auditory thalamus to the auditory cortex (Purves 2001).

2.6 The vascular baroreflex

Another system that requires transduction of mechanical stimuli is the vascular baroreflex, responsible for balancing short term changes in blood pressure. Blood pressure changes are detected by mechanosensitive afferents innervating the walls of arterial vessels in the carotid sinus and in the aortic arch (**Figure 3**). The mechanosensitive cells are sensory neurons that lie in the vessel walls as free nerve endings (Rees 1967). The sensory neurons that innervate the carotid sinus are part of the glossopharyngeal nerve and their cell bodies are located in the inferior glossopharyngeal ganglion (petrosal ganglion). The sensory neurons that innervate the aortic arch are part of the vagus nerve and their cell bodies are located in the inferior ganglion of the vagus nerve (nodose ganglion). The mainly slowly adapting baroreceptors respond to changes in mean pressure in the artery. However, the frequency of an applied sinusoidal stimulus is also positively related to receptor activity (Koushanpour 1991). The nerve fibers are either myelinated or unmyelinated (A- or C-fibers) (Fidone and Sato 1969); the C-fibers are reported to have lower firing thresholds, and lower response levels than A-fibers (Fidone and Sato 1969; Brown, Saum et al. 1976; Yao and Thoren 1983; Coleridge, Coleridge et al. 1987).

The molecular identity of the transduction complex is not known, but it has been suggested that the alpha subunit of the ENaC ion channel may play a role (Drummond, Welsh et al. 2001), again a homologue of a *C. elegans* mechanotransduction protein. The function of ASIC2, another member of the DEG / ENaC superfamily was studied in *Asic2*$^{-/-}$ mice, and these mice showed reduced baroreflex activity (Lu, Ma et al. 2009).

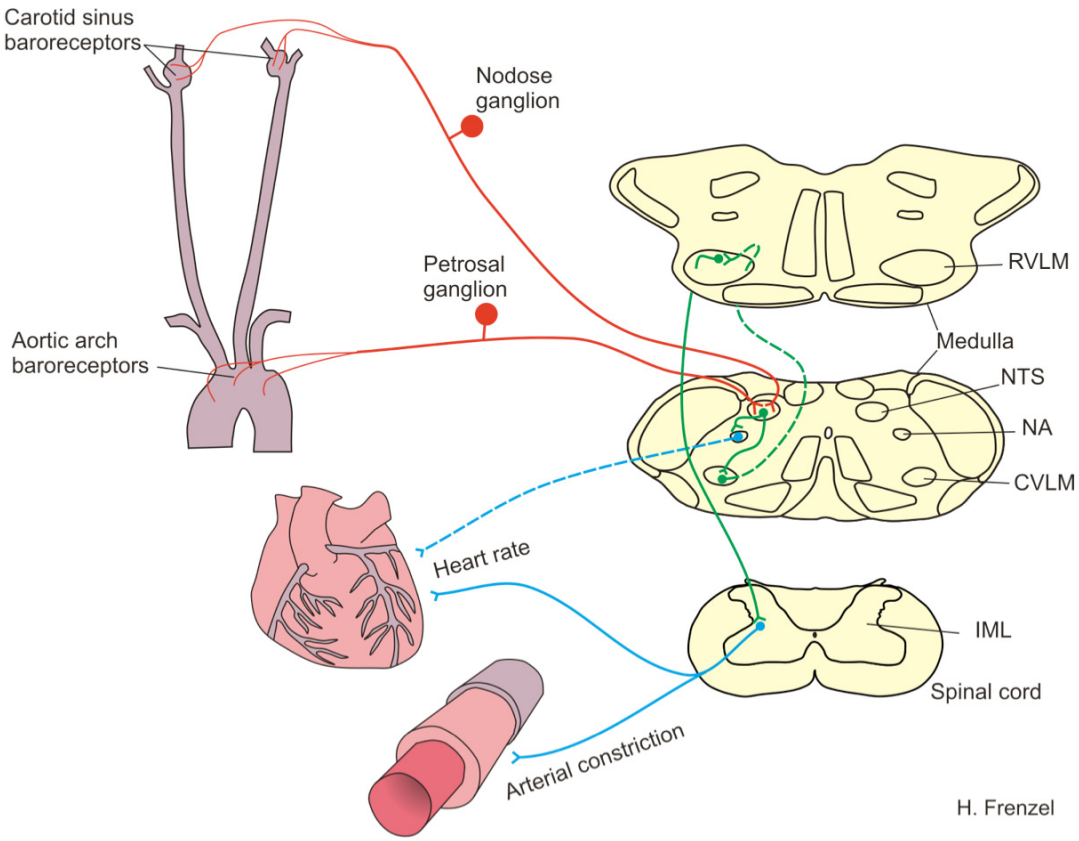

Figure 3: Schematic representation of the vascular baroreflex. See text for description. Red lines depict sensory neurons, green lines interneurons and blue lines efferent neurons. Solid lines depict excitory neurons, dashed lines inhibitory neurons. NTS = nucleus tractus solitarius; NA = nucleus ambiguous; CVLM = caudal ventrolateral medulla; RVLM = rostral ventrolateral medulla; IML = intermediolateral cell column.

.

The baroreceptor neurons from the carotid sinus and from the aortic arch both terminate in the same structure of the medulla, the nucleus tractus solitarius (NTS). From here, there are two effector pathways leading to a regulation of blood pressure. Neurons from the NTS activate neurons in the caudal ventrolateral medulla (CVLM), which in turn inhibit neurons in the rostral ventrolateral medulla (RVLM). Since the neurons of the RVLM directly activate neurons of the sympathetic system, inhibition of the RVLM by the baroreceptor reflex leads to an inhibition of the sympathetic system. A decrease in sympathetic tone leads to a decrease in both heart rate and arterial constriction and thus a decrease in blood pressure. In the second pathway, NTS neurons activate neurons in the nucleus ambiguus that, in turn, control the parasympathetic system. An increase in

parasympathetic tone leads to a reduction of the heart rate via the vagal nerve (Seller 1991).

The function of the baroreflex is thought to be responsible for two types of oscillatory variations in heart rate (Cohen and Taylor 2002). High frequency (HF) oscillations at ~0.25 Hz are interpreted as the baroreceptor response to respiration. Oscillations at low frequencies (LF) at ~0.1 Hz, the so called Mayer waves, are thought to be the result of resonance within the baroreflex system itself.

2.7 Aims

The aim of this study was to explore the genetics of various mechanosensory traits, particularly of touch sensitivity. As mentioned before, there are no known genes that specifically influence touch sensitivity in humans. It is also not known if there is a genetic component in the variation of touch sensitivity traits that can be observed in humans or if all the variation arises from environmental influences that individuals encounter throughout their lives. To address this question a classical twin study was performed, where the similarities of touch sensitivity between monozygotic and dizygotic twin pairs were analyzed and heritability values determined. Heritable components had been shown before for the other sensory systems described above and those were also included in the twin study.

Furthermore, we hypothesized that there might be common genetic factors that influence different mechanosensory systems. It was shown for *Drosophila melanogaster* that the same mechanotransduction channel is involved in tactile sensing and in hearing (Eberl, Hardy et al. 2000; Kim, Chung et al. 2003). However, it has not been addressed so far if different mechanosensory mechanisms in vertebrates evolved independent of each other or if they might have evolved from one primordial system.

When we assume common genetic factors, we also expect correlation on a phenotypic level. This was investigated by comparing parameters of different mechanosensory traits in healthy individuals. For the analysis of phenotypic correlations the datasets from the twin study were used in addition to another cohort of healthy individuals.

In contrast to touch sensitivity there are a large number of genes known to cause deafness or hearing impairment when mutated. Therefore, it is possible to specifically ask whether there are common genes involved in touch and hearing by assessing touch sensitivity in cohorts of individuals that suffer from a diagnosed, or suspected, genetic hearing impairment or deafness to see if they also show altered touch sensitivity. Here we focused on patients that suffer from the Usher Syndrome as some of the affected genes (*Cadherin23*, *Protocadherin15*, *Myosin7A*) have been shown to be part of the mechanotransduction complex of the hair cell, as described above.

In another project carried out in collaboration with Dr. Stefan Lechner we screened for genes that are involved in mechanotransduction in the cutaneous sensory neurons in the mouse. Here we made use of recent findings about the development of mechanosensory neurons. These neurons start to acquire their mechanosensitivity at a certain embryonic stage (E13.5) and it can be assumed that the expression of genes relevant for mechanosensitivity is upregulated at this stage. Therefore, we screened for genes that are candidates for being involved in the mechanotransduction process by comparing expression levels on the mRNA level between mechanoinsensitive and mechanosensitive developmental stages.

3. Material and Methods

3.1 Materials

3.1.1 Technical equipment

Althen DI-720-USB, DI-205 data acquisition device
Applied Biosystems Prism 7000 Sequence Detection System
BDK Laminar Flow Hood
Forma Scientific Steri-Cult 200 Incubator
General Electric Dinamap Pro 100 blood pressure monitor
Heraeus Biofuge 13
Heraeus Megafuge 1.0
Medis Cardioscreen ECG monitor
Medoc advanced medical sytems TSA-II thermal sensory analyzer
Ohmeda Finapres 2300 blood pressure monitor
Pharmacia Ultrospec 1000 Spectrophotometer
WR Medical Electronics CASEIV System

3.1.2 Software

7000 System Software, Applied biosystems
GraphPad Prism4, GraphPad software
MxGui, Version 1.7.03, Dept. of Psychiatry, MCV, VCU, Gaohong Xie
PV-Wave, Visual Numerics
TestWorks, WR Medical Electronics
Universal Probe Library Assay Design Center, Roche
WinDaq Pro+, DataQ Instruments
Wintsa, Medoc advanced medical systems

3.1.3 Chemicals and reagents

DMEM-F12, Invitrogen
Glucose, Sigma-Aldrich
Laminin, Invitrogen
L-Glutamin, Sigma-Aldrich
NT-3, Alomone
PBS Ca^{2+} and Mg^{2+}-free, Gibco
Penicilln-Streptomycin, Gibco
Poly-L-lsyin, Sigma-Aldrich
Random hexamers, Invitrogen
RNAlater, Qiagen
Trypsin, Invitrogen

3.1.4 Molecular biology kits, enzymes

Mouse Genome 430 2.0 Array, Affymetrix
RNase free DNase Set, Qiagen
RNeasy Mini Kit, Qiagen
SuperscriptII Reverse Transciptase, Invitrogen
TaqMan Universal PCR Master Mix, Applied Biosysytems

3.1.5 Primers

3.1.5.1 Primers – Screening of candidate genes

Table 1: Primers used in the candidate expression screen

Gene	Accession No.	5' - Primer	3' - Primer	Universal probe library No.
Enac alpha	NM_011324	5' ccaagggtgtagagttctgtga	5' agaaggcagcctgcagttta	45
Enac beta	NM_011325	5' ggcctctgaggattggatct	5' tgagcttgacaataccccttcc	93
Enac gamma	NM_011326	5' tcttcgatgggatgtcctgt	5' cgtgtagcagttcccatacatt	31
Asic1a	NM_009597	5' ctggccctgctcaacaac	5' ggaagttggccttgtcctg	78
Asic1b	AB208022	5' gagctggatgagggtgactc	5' gagctggatgagggtgactc	49
Asic1		5' ggcttccagacgtttgtgtc	5' tggtaacagcattgcaggtg	68
Asic2a	NM_007384	5' agagcccagggattggag	5' ctagctctggcctctcctaactac	104
Asic2b	NM_001034013	5' cctgaagccagttgcagaac	5' atctggatgctggaaggttg	45
Asic2		5' ctttctgcacccctgagc	5' tgtccttttctgccagtaagc	21
Asic3	NM_183000	5' ccctgtggacctgagaactt	5' cccttaggagtggtgagcag	104
Asic4	NM_183022	5' cctgacatggtagacatcctca	5' cacttcccatagcgagtatagacc	49
Trpc1	NM_011643	5' tgaacttagtgctgacttaaaggaac	5' cgggctagctcttcataatca	10
Trpc2	NM_011644	5' tgctgcaacttgtggagaga	5' aacgggtgttagtgccacat	2
Trpc3	NM_019510	5' ttaattatggtctgggttcttgg	5' cacaactgcacgatgtactcc	11
Trpc4	NM_016984	5' aaggaagccagaaagcttcg	5' ccaggttcctcatcacctct	9
Trpc5	NM_009428	5' gcaatcaaatatcaccagaaagag	5' gccatcgtaccacaaggtg	102
Trpc6	NM_013838	5' gcagctgttcaggatgaaaac	5' ttcagcccatatcatgccta	58
Trpc7	NM_012035	5' gtggcctacttcacctacgc	5' acagcccttccgagatgat	49
Trpm1	NM_018752	5' catcattatgcgcctcagc	5' cgtcgctaaggaacaacttca	63
Trpm2	NM_138301	5' ttcacagacctgagccagaa	5' gacactggagggcgtgtc	60
Trpm3	NM_001035239	5' ctccggatgagaaagaactca	5' ttgttcttgaactccaagctga	69
Trpm4	NM_175130	5' gaggcccttagccactctg	5' catccaccaggaacactcag	16
Trpm5	NM_020277	5' ggatcaagtgtctggaatcaca	5' tcctgcaaccacagttctga	1
Trpm6	NM_153417	5' cacaagccagtgaccaccta	5' ttccatgtgggggttttatc	5
Trpm7	NM_021450	5' gagacgctttccgataggtg	5' ctatccaggatttctgggacat	25

Trpm8	NM_134252	5’ tcagatacacagagatccttctgc	5’ ggctccctcgaaggacat	94
Trpv1	NM_001001445	5’ accacggctgcttactatcg	5’ tccccaacggtgttattcag	97
Trpv2	NM_011706	5’ caccatagttgcctaccacca	5’ gtcgcttttgatgagggaat	71
Trpv3	NM_145099	5’ atgggctcacaccactgc	5’ ggctgaggatgtacttcagga	71
Trpv4	NM_022017	5’ ccaccccagtgacaacaag	5’ ggagctttggggctctgt	25
Trpv5	NM_001007572	5’ gagagggacgagctctgga	5’ acaggaaacgaggcattttc	67
Trpv6	NM_022413	5’ gagctctggagagcacaggt	5’ acgaggtagcttccgctcta	49
Pkd1	NM_013630	5’ gccatccagcacttcctagt	5’ gagaagccgatccacacatc	92
Pkd1l1	XM_126005	5’ tgtggatgaggaccagcac	5’ agctctgggttagcctggat	76
Pkd1l2	NM_029686	5’ gacaccgtaccagaggaggt	5’ gtcagtgatcgtggccaaa	27
Pkd1l3	NM_001039700	5’ ccagctacggagtgagtttga	5’ gactctgctggcaaaatgct	67
Pkd2	NM_008861	5’ tgaagagacgagaggtgttagga	5’ cactgtcccgacccagtc	42
Pkd2l1	NM_181422	5’ ctggacctggtggtcatctt	5’ gggttcggaatatgtggaaa	76
Pkd2l2	NM_016927	5’ gccagaagagaaggctttga	5’ gcctttccttccatcttttca	34
Pkdrej	NM_011105	5’ ttcctcgtacaaccatgcaa	5’ acttgggcttgtagctcctg	2
Trpml1	NM_053177	5’ gcgcctatgacaccatcaa	5’ taggcctggagctcactctt	79
Trpml2	NM_026656	5’ ttgccctcaaaggaattgac	5’ ctgtgttgtcgaaggtaatcgt	96
Trpml3	NM_134160	5’ attgcttttgcggatggat	5’ ctgttcagggaacggaactt	107
Trpa1	NM_177781	5’ ccatgacctggcagaatacc	5’ tggagagcgtccttcagaat	32
Stomatin	NM_013515	5’ ccagtgcagctccagagag	5’ cgcattcatttccccttc	29
Slp1	NM_026942	5’ gatccagatggagaagctcaag	5’ ggtcacgtcattgatctcca	17
Slp2	NM_023231	5’ tcacgagtatggtggctcag	5’ tctgctctggctggagttct	45
Slp3	NM_153156	5’ cttgtaacattcctccacaagaga	5’ gacggcgctgtagattctgt	58
Cdh23	NM_023370	5’ agccggcctacttcgtgt	5’ gaaacaacactgtggctcctg	67
Pcdh15	NM_023115	5’ tcgtcttagtaagctaccgacagtt	5’ gaattcttgcggtcttcgtg	64
Sans	NM_176847	5’ gcctcggaagaagatcctg	5’ ttcatttggtctgaggagagg	50
Ush2a	NM_021408	5’ aggaggcagcatacggttac	5’ cgcgagtgtgaagctgtagt	19
Gpr98	NM_054053	5’ tgtatgatgtcaagacccaagg	5’ tccctgggcatcaagtagag	18
Ush3a	NM_153384.2	5’ cctttccggttctcatgct	5’ cggtggactttcacttcagag	5
Pres	NM_030727	5’ ggtctcgggcataagcact	5’ agcatggcgaaggctaag	60

Myo15	NM_010862	5' ggctccctgctcaactaaga	5' ccagcgatctcacagttcc	75
Tmc1	NM_028953	5' ggagaacaaaatgcgaaacaa	5' gcacctccaggacatttgat	49
Otof	NM_031875	5' agaggagccccaaagacaag	5' agctgaatggctaggtggtc	67
Strc	NM_080459	5' agctctggtagctgggattg	5' ccgcacagtttggtacagg	42
Myo3a	NM_148413	5' acacctccgtaggaacacca	5' gttgctgttcacaagcaatca	92
Whrn	NM_001008791	5' ggtgcaaaccagcactttg	5' tgagagctgagagagtgtggag	22
Espn	NM_019585	5' tgcctggagacgagacatt	5' tcctcttttcgcttctgctc	25
Myo6	NM_001039546	5' gaggaagccggaagcact	5' gcacagtattccagggatgg	83
Kcnq4	XM_900381	5' ggatgccagcagctaatctc	5' ggcttgtgtccgtggagta	64
Pouf3	NM_138945	5' ccccgtactgcaagaacc	5' catcaaagcttccaaatatattaccc	19
Wfs1	NM_011716	5' tggagatccccttgaagaa	5' ggcaaggcgtaggtagtgtt	19
Cldn14	NM_019500	5' cctgcttgcctgatgtatga	5' cccagggatggatctggt	4
Myh14	NM_028021	5' taaactggcccaggcaga	5' cagcttgccagagaggatg	15
Myh9	NM_022410	5' gtccatgccggacaacac	5' ggtgaagtcggtcacattgat	10
Tfcp2l3	NM_026496	5' ccacagagcatactgccaga	5' tctcttcatcccggatttttc	32
Triobp	NM_001024716	5' ggctctcaggaaaactgtgc	5' tttattgcagtcggagagctt	45
Tmhs	NM_026571	5' tccttcctggcttttgtgtt	5' cggttgcttcagacttcctc	52
Crym	NM_016669	5' ggggctcacatcaatgct	5' gctcgtcatccagttctcg	40
Diaph1	NM_007858	5' aagcagattgcggacgtg	5' gcatccttcacaaagctggt	33
Dfna5	NM_018769	5' ggggatccagaccaagactat	5' acattggtgtctgtggtgaca	88
Eya4	NM_010167	5' cccaggacctaaatgagcaa	5' tttccatagacctggaattctga	104
Tmie	NM_146260	5' tcccttggggtgctttct	5' ggggtggctctcaactatca	95
Actg1	NM_009609	5' gagcacgctgtagatgagaaag	5' gatcactcagtggtgctcaca	64
Myo1a	XM_483962	5' aggtcaaccgtggcaatg	5' agaatcacgtgccccttg	63
Hprt1	NM_013556	5' tcctcctcagaccgctttt	5' cctggttcatcatcgctaatc	95
Nse	NM_013509	5' cactaacgtgggggatgaa	5' caccagctccaaggcttc	80
Mtap2	NM_001039934	5' tctaaagaacatccgtcacagg	5' ttccttgaaatccagttttacactc	77

3.1.5.2 Primers – realtime PCR confirmation of candidates from expression chip experiment

Table 2: Primers used in the candidate expression screen

Gene	Accession No.	5' - Primer	3' - Primer	Universal probe library No.
Abca8a	NM_153145	5' cctggttccacctgctacat	5' atttcagaaaattcacgattctca	75
Ogn	NM_008760	5' ggaattaaagcaaacacattcaaa	5' tttctggtaaattaggaggcaca	85
1500015o10rik	NM_024283	5' tccagatggcataagtggaa	5' attagtctttgacgggacaggt	50
Cdh1	NM_009864	5' atcctcgccctgctgatt	5' accaccgttctcctccgta	18
Aqp4	NM_009700	5' gttggaggattgggagtcac	5' tgaacaccaactggaaagtgat	22
Gm10672	AK140299	5' tcaaggatcaaagggaatcg	5' ccaaggcggacagaataaag	81
Abhd3	NM_134130	5' ggtgtgtggtttttaataacagagg	5' gcagtaagtccgtggtgtca	2
2900078e11rik	AK013801	5' ttctccagtgaaagattacaaacg	5' aatgcgtttggcataacaga	22
Mctp2	NM_001024703	5' acagccaggaaagcacagac	5' tccccttttctcagactcct	69
2900092e17rik	NM_030240	5' ccagacagcggaagtactga	5' agcacaaaacagtctgcacct	51
Mal	NM_010762	5' gacttcctggatcacactgga	5' gcttccagaactgaggcact	5
Popcd3	NM_024286	5' cctgagtgggattcgctaag	5' atatcggcaatcggtgtctg	6
Atp1a3	NM_144921	5' ccgctcatcagtctgaacg	5' ggcgagctcttgtcatctttt	103
Itgbl1	NM_145467	5' ccagatggcaaagtctgtagc	5' tcatggcaagaacattcacc	6
Cntnap2	NM_025771	5' cgtgcacattcagggtga	5' tgtcctgggttatatgggaaa	31
Nkain2	NM_001025286	5' ttgggttgtttggaactattca	5' cagcctccaaatagaagcaga	85
Ugt1a1	NM_201645	5' ccttaaaactgtcatcaacaacaag	5' gccaggtccagaggctctat	19
Tmem16d	NM_178773	5' cgcttatgactgggatctgatt	5' gcttcaaactggggtcgtat	69
Fads6	NM_178035	5' attcggccactccctcat	5' ttcgacaccaagggcttc	103
Susd2	NM_027890	5' tgctgctctcaacaataccag	5' aggaacagagtccatcctgtg	62
Adam23	NM_011780	5' gactacgtggagatccactatgaa	5' ttccgtggtagtaacagtgctc	38
Pcdha1	NM_054072	5' actctgcctcgctaagagca	5' tactgttggccactgctgat	79
Pcdhb9	NM_053134	5' ctgggaaaggctctgtaacg	5' tcagctctggggcattgt	77
Ccdc109b	NM_025779	5' agtggagccacaggatgaa	5' aaactggcagcgctctttt	53
4732435n03rik	NM_172753	5' tcagtataaccccggcgtag	5' cccgtttccttctttatgacc	56

Slc41a2	NM_177388	5’ gtttacacgccagttatcaacg	5’ tggaggtaggtagaaatcctgct	49
Gramd1b	NM_172768	5’ gtgaagcccctgtttcgtt	5’ gaaccatccccctccatc	25
Bb146404	NM_178908	5’ ctgcaggcccagtctcag	5’ gcgtgcataacaagtggtca	55
Lrrtm2	NM_178005	5’ tgagtctgacaacctcgactg	5’ atggccacttgaaatgtaagc	75
Opmcl	NM_177906	5’ atgccagcatcaccctgtat	5’ ctctggaggccgagtttaca	1
Jakmip1	NM_178394	5’ catcgatgacctctctctgga	5’ tcttccctcgatgcctctt	40
Frmd3	NM_172869	5’ attcgactgctggacgactc	5’ gaaactgccctttcgtttcc	51
Hs3st5	NM_001081208	5’ ccagagttgggagcttgg	5’ caccaccaaatcgactttca	19
Tln2	NM_001081242	5’ gcctctcaccacggtcaa	5’ actgaatagtagctctcttggtactcc	47
Slc24a2	NM_172426	5’ tctggatcaccttacctgacg	5’ tggagccgaagaatgtgata	38
Abca8b	NM_013851	5’ tggagcccttatagtgtctgg	5’ ctcaggatgcccatcagaa	38
Lsamp	NM_175548	5’ cccaagacctcccaagttta	5’ tcacagtgacatccgaggag	31
Zfp179	NM_009548	5’ cagcaacagttgggttctcc	5’ cagcttggggaatgatgc	42
C330002i19rik	NM_001081378	5’ agaatggagccaactgcaac	5’ gtgcccctctttagatgctg	85
Cd55	NM_010016	5’ actgttgattgggacgatgag	5’ tggtggctctggacaatgta	47
Pcnxl2	NM_175561	5’ ggaagccagagggagagg	5’ tgtgaatctgcctgttattgct	71
6030405A18RIK	NM_177854	5’ cctgtattcccttgagtctcctc	5’ atcacttgtccacggagacc	71
TMEM25	NM_027865	5’ cgctcccaacatgagctt	5’ tggtatttggctccaacctg	32
BEAN	XM_486154	5’ ggatgtggatgtcacagtgc	5’ ggtcccatgcattcctcat	22
2210419i08rik	AK134433	5’ aatgttgggcctgattttctac	5’ tttttgaaccaggaaaaacttgt	103
Aqp11	NM_175105	5’ accaagcttcgcatccac	5’ cctgtgaggctccctcct	71
Bc062109	NM_182841	5’ ttaccctctgcgtagtcctgtat	5’ ccaggaagcacatgaccag	71
Endod1	NM_028013	5’ ttcgccactctgtacagtcct	5’ tgtcagggtcatcaatctgc	66
Slc7a4	NM_144852	5’ ttgtggcagttggctctatct	5’ cggcagggagaagaggtt	15
Bb181834	XM_890874	5’ gctgatgtttctcccatagtgtc	5’ tcgccagttgccttctacat	4
Hspa1a	NM_010479	5’ ggccagggctggattact	5’ gcaaccaccatgcaagatta	84
Sspn	NM_010656	5’ agcccctccctgctagtc	5’ agtccaaggtaagccaccac	26
Slc7a7	NM_011405	5’ ccagggtcctgtgtttgc	5’ atgggggtgtgacttcagc	68
Mmp24	NM_010808	5’ ggcatccagaagatttacgg	5’ gtagggaggggccttgtg	110
Slc10a6	NM_029415	5’ agcaacggtcattctcaagg	5’ cttttgccaggaccatgc	63

Cldn1	NM_016674	5' actccttgctgaatctgaacagt	5' ggacacaaagattgcgatcag	18
Dgke	NM_019505	5' gtattctgcaggcagcagtg	5' gtcttctggcaccaaatgc	71
Lglas3bp	NM_011150	5' gctccaggactgccttca	5' cattgcctggcctagaagc	27
Slc7a2	NM_001044740	5' agtcggcttcccttgtgag	5' tactcaagcccaggatgagg	72
Slc27a1	NM_011977	5' gacaagctggatcaggcaag	5' gaggccacagaggctgttc	1
Lrrc3	NM_145152	5' tggcttcagctgccactta	5' atcagacggctcacagacct	64
A2m	NM_175628	5' tgaggaggcggtaaaagaag	5' tggcactctgggtttctga	93
Epb4.1l1	NM_013510	5' tgtctatagagaaacagacccatcc	5' acgccagcatctcttcaca	91
Atxn1	NM_009124	5' tcttgagaagaaacaaacctgct	5' tttctttcgcagagaggttagaa	53
Cyp2j9	NM_028979	5' cagtcactcttctccgaaaacat	5' cagtcactcttctccgaaaacat	12
Slc16a9	NM_025807	5' cctcggcctgatttcaac	5' agcaggcagccatctagg	89
5930434b04rik	NM_029862	5' gaagagggcatgacttggtg	5' agtccagagatggcacagga	76
Itgb4	NM_133663	5' agtgtgatctgtgacgtgtgc	5' acagcgagctgatcgaactt	100
Lrrc24	NM_198119	5' cgagcaggaggctctcagt	5' agggaatggacctggatagg	98
Mtm1	NM_019926	5' gtttgagatccttgtaaaacatgc	5' cacccatccacgttaaacttc	6
Cyp2u1	NM_027816	5' accacgaccaactctctgct	5' cctttcaatttcttcatgaacctt	40
Paqr6	NM_198410	5' aaacaggtcaacgtggaggta	5' atgccttcttcccaaaacac	49
Adcy9	NM_009624	5' ttggggcaatcttggtgt	5' cagagccagtgaacatggtg	4
Slc25a32	NM_172402	5' ccattagagtacctcgtctcagc	5' gggtttgtaatgcacagagtca	78
Tmem100	NM_026433	5' tggcctctctggtaatggac	5' agatttggaaagcctggtca	100
Npal2	NM_145469	5' ggaaaggaatgaagcacattg	5' catgcctgagacagccttc	15
Atp9b	NM_015805	5' ggtttgtctgtggaggatgg	5' catttattagccaatcacagcaac	6
Slitrk4	NM_178740	5' gcgagtcggaaagcagtg	5' cacggtctttgagcagcttt	50
Yipf3	NM_145353	5' tcctcagaccctactttgatgtg	5' ccatcttgatagggatcatgg	92
9430079b08rik	NM_027534	5' tccaagtttgccataagagga	5' gtacacattgtacggcttcacc	29
Sfxn5	NM_178639	5' gcttcctccaatcgtcatgt	5' cacgaggctatgcacaggta	67
Slc5a6	NM_177870	5' gcctgggaatggcctatatt	5' ctccaaccatgccaaagatac	1
Bc038479	NM_153803	5’aaggctgatgttaccagctatga	5’gcttggtgtatttggcagtg	19
Fxyd2	NM_007503	5’cctcctgcctcagatcctta	5’gaacagggagtggggtgtta	60
Tmem130	NM_177735	5’gtgtgccagccattagcc	5’tgtttcccacaagggactct	52

3.1.6 Animals

All mice were C57BL6/6N, obtained from Charles River WIGA. Mice were sacrificed by CO_2 inhalation. Developmental stages were determined using the Edinburgh Mouse Atlas Project (EMAP), MRC.

3.2 Methods

All psychophysical and physiological testing procedures were approved by the *Ethikkommission* of the *Charité – Universitätsmedizin Berlin*. All participants gave their written consent.

3.2.1 Tactile acuity test

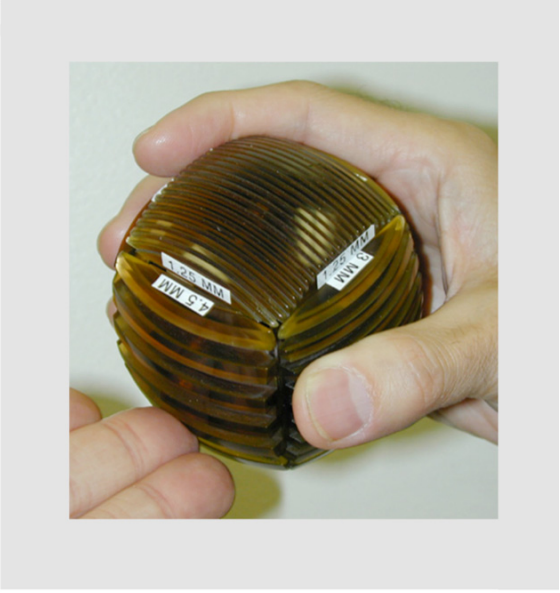

Figure 3: The Tactile Acuity Cube

Tactile acuity was determined using the Tactile Acuity Cube (MyNeurolab.com / Leica Microsystems) (**Figure 3**). In the tactile acuity test a transformed-rule up and down

method was applied (Zwislocki and Relkin 2001). Test persons put their hand, with the palmar surface looking upward, on a table and (sighted) test persons were blindfolded. The Tactile Acuity Cube was applied for one second to the fingerpad, in a way that the cube exerts its whole weight on the finger. Test persons had to determine if the orientation of the gratings on the cube was parallel or perpendicular to the fingers starting with the widest grating width. Each grating width was tested two times and if two answers were correct the next, smaller width was tested; this was continued until the test person answered incorrectly. The grating width was then increased stepwise again until the two orientations of a width were determined correctly again. Thirteen of these turning points were determined and the mean of the last ten taken as the threshold. The threshold corresponds to the grating width where the probability of a correct answer is 0.707. Thresholds were determined for the little finger and the index finger and the mean of this threshold taken as the tactile acuity.

3.2.2 Vibration detection threshold test

Figure 4: The vibration stimulator of the CASEIV system

Vibration detection threshold was determined using the CASEIV system (WR Medical Electronics), according to the manufacturer instructions. In the vibration detection test a transformed-rule up and down method was applied (Zwislocki and Relkin 2001) in connection with a two-interval forced choice test. A vibration stimulator was applied

below the nail of the little finger (**Figure 4**). To prevent possible auditory detection of the vibration stimulator, (normal hearing) test persons wore headphones, which produced a low, continuous tone during the test. A sinusoidal 125 Hz vibration was applied during one of two periods indicated to the test persons. Then they had to determine in which period the vibration was applied. A step towards the next smallest amplitude was done when the test person responded correctly six times in maximum of eight trials, otherwise a step to the next biggest amplitude was done. Eight such turning points were determined. The threshold corresponds to the vibration amplitude at which approximately 75 % of the answers are correct (Dyck, Zimmerman et al. 1978). The amplitude magnitude steps are just noticable differences (JNDs) that have been previously determined and resemble a logarithmic representation of the amplitude in µm.

3.2.3 Temperature sensitivity test

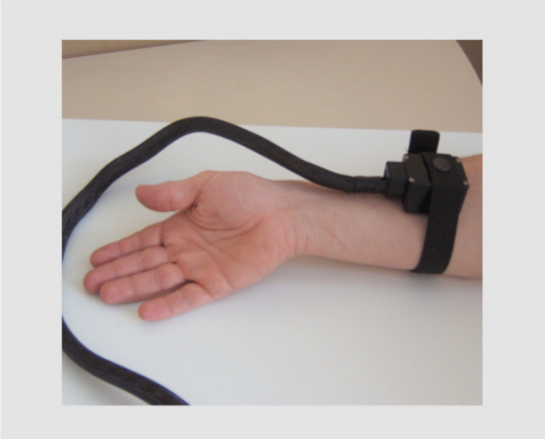

Figure 5: The thermode of the TSA-II system applied to the volar forearm

Temperature sensitivity was determined using the TSA-II System (Medoc advanced medical systems) according to manufacturer instructions. The thresholds were determined using the ascending method of limits. A peltier thermode was placed in the middle of the volar forearm (**Figure 5**). The baseline temperature for all four tests was 32° C and the temperature change rate was 0.5 °C / s. In the temperature change detection tests the test person indicated when they felt cooling or warming respectively. The mean of four

thresholds was calculated. In the temperature pain threshold tests, the test person indicated when a rising or falling temperature became painful. Here the mean of three thresholds was calculated.

3.2.4 Audiometry

Audiometry was carried out in the Klinik für Audiologie und Phoniatrie, Charité – Universitätsmedizin Berlin employing the standard procedures for clinical use. For hearing acuity the pure tone thresholds in decibels (dB) at 0.5, 1, 2 and 4 kHz were determined using a ST36 Audiometer (Maico) and the mean calculated. The otoacoustic emissions were measured using a OAE (Otodynamics). Otoacoustic emissions were evoked by 1 ms clicks spanning a frequency range from 0 – 6 kHz. The measured parameters were the overall intensity of the emissions in dB and the reproducibility of the frequency distribution of consecutively evoked emissions in %.

3.2.5 Baroreflex function measurement

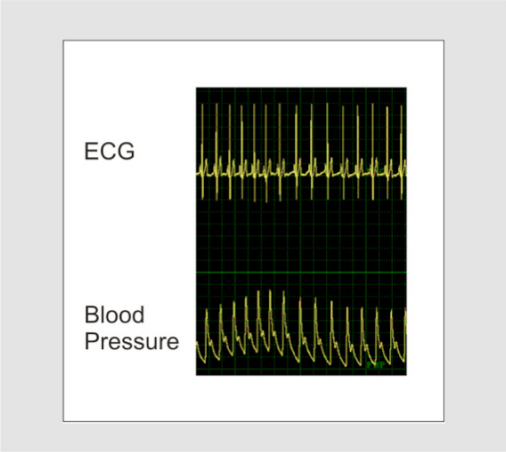

Figure 6: Screenshot of simultaneous recording of blood pressure and ECG.

To determine the baroreflex at rest, test persons were in a supine position. Blood pressure (BP) was monitored with a Dinamap Pro 100 (General Electric) until a steady BP level was reached. Two Dinamap Blood pressure measurements were then recorded (as well as two more after the whole procedure) and used later for correction of baroreflex sensitivity

for BP. Subsequently BP was measured for 10 min in the middle finger continuously using a Finapres 2300 (Ohmeda), as well as the electrocardiogram (ECG) using a Cardioscreen (Medis). BP and ECG were recorded simultaneously using the data acquisition devices DI-720-USB and DI-205 (Althen, Meß- und Sensortechnik) and the WinDaq Pro+ (DataQ Instruments) software with a sampling rate of 1 kHz per channel (**Figure 6**). These data were transferred into beat to beat files (as determined by the RR – interval) and analyzed using the PV-Wave software (Visual Numerics). Baroreflex sensitivity was determined by two methods. Firstly the sequence technique in which sequences of 3 coupled minimum steps of 0.5 mmHg BP changes and 5 ms RR – interval changes with minimum correlation coefficients of 0.85 were detected and their slopes taken as the baroreflex sensitivity in ms / mmHg. Secondly cross-spectral analysis, where variations in BP and the RR - intervals were analyzed in the frequency domain. Baroreflex sensitivity was calculated as the mean value of the transfer function in the low frequency band (0.04 – 0.15 Hz) and in the high frequency band (0.15 – 0.4 Hz) also in ms / mmHG. For both methods a period of 5 min was analyzed (Linden and Diehl 1996; Soc.Pacing-Electrophysiology 1996).

3.2.6 Heritability analysis

Narrow sense heritability (h^2) estimates were performed by structural equation modeling using the Mx software developed by Neale (Neale 2004). The ACE model used is depicted in **Figure 12**. Six elements are described by the model: the variance of the trait in twin 1 and in twin 2 and the covariance of the trait in twin 1 and twin 2, each separately for MZ and DZ twins. These elements are described as follows:

$a^2*c^2*e^2$ = variance of trait in MZ twin 1
$a^2*c^2*e^2$ = variance of trait in MZ twin 2
$a^2*c^2*e^2$ = variance of trait in DZ twin 1
$a^2*c^2*e^2$ = variance of trait in DZ twin 2
a^2*c^2 = covariance of trait in MZ twin 1 and twin 2
$0.5a^2*c^2$ = covariance of trait in DZ twin 2 and twin 2

where a^2 is the variance component determined by additive genetic effects, c^2 is the variance component determined by common environment effects and e^2 is the variance component determined by unique environment effects. a^2, c^2 and e^2 are the parameters estimated by maximum likelihood estimation. a^2 is identical to the heritability h^2.
The ACE model, as well as the AE and the CE sub-models were tested and the best fitting model selected according to the χ^2 Value. The script used was provided by the SGDP (MRC), King's College, London. Transformation of datasets was conducted, if necessary, so that a normality test was passed (Kolgomorov-Smirnov test).
Zygosity tests were performed in the laboratory of Prof. Norbert Hübner (MDC - Berlin). 11 Satellite DNA markers (d16s2426, d17s1806, d3s1578, d10s1430, d17s790, d18s858, d18s57, d11s2000, d12s79, d1s238, d3s1267) were amplified in PCR reactions and compared for size differences.

3.2.7 Realtime PCR

3.2.7.1 Realtime PCR - dissected DRGs

Dissected DRGs of the relevant developmental stages were collected in the RNA stabilization reagent (Qiagen) RNAlater. Total RNA was extracted and DNA digested using the RNeasy Mini Kit (Qiagen), including the RNase free DNase set (Qiagen). In this step, the lowest amount (350 µl) of the first buffer (RLT) was used. DRGs were disrupted using a glass pestle and mortar. RNA was quantified using an Ultrospec 1000 Spectrophotometer (Pharmacia Biotech). 2 µg of RNA per reaction was reverse transcribed using the SuperscriptII Reverse Transciptase (Invitrogen) using 3 µl of 50µM random-hexamers. From the resulting ~20 µl of cDNA, 0.5 µl was taken for each PCR. PCRs were performed in 20 µl reactions in MicroAmp optical 96-well reaction plates in an Abi Prism 7000 Sequence Detection System (Applied Biosysytems). Probes from the Universal Probe Library (Roche) were used and primers designed using the Universal Probe Library Assay Design Center. The TaqMan Universal PCR Master Mix (Applied Biosysytems) was used. All PCRs were conducted in duplicate and the mean threshold

taken as n = 1. When thresholds in duplicates differed by more than one cycle, this measurement was not considered. All mRNA levels were normalized to *Hprt1* mRNA levels.

3.2.7.2 Realtime PCR - cultured DRG cells

DRGs from all spinal segments of four E 12.5 embryos were dissected, collected in Ca^{2+} and Mg^{2+}-free PBS (Gibco) and treated with trypsin (0.05%, Invitrogen) for 12–20 min at 37°C. Digested DRGs were washed twice with medium (DMEM-F12 (Invitrogen), supplemented with L-glutamine (2 mM, Sigma-Aldrich), glucose (8 mg/ml, Sigma-Aldrich), penicilin (200 U/ml)–streptomycin (200 mg/ml) 5% fetal horse serum), and triturated using fire-polished Pasteur pipettes and seeded in a droplet of growth medium including 10ng / ml NT-3 on a glass coverslip precoated with poly-L-lysin (20 mg/cm2, Sigma-Aldrich) and laminin (4 mg/cm2, Invitrogen). Cells were seeded on 12 coverslips in a 12 – well plate (Falcon). Coverslips were kept for 4 h at 37°C in a humidified 5% incubator, for 24 h cultures, growth medium was replaced after 4 h.

For harvesting, cultures were washed twice using PBS and the PBS removed, coverslips were rinsed with 350 µl RLT buffer from the RNeasy Mini Kit (Qiagen). The same 350 µl RLT buffer was used for six coverslips. The rest of the procedure was as described for the dissected DRGs except that the amount of RNA used for reverse transcription was less than 2 µg, here the maximum possible volume was used (4 µl) in the reverse transcription step using the SuperscriptII Reverse Transciptase Kit (Invitrogen). All mRNA levels were normalized to the mRNA levels of *Mtap2* and *Nse*.

3.2.8 Gene expression chip analysis

DRGs of the respective stages were collected in RNAlater (Qiagen) and RNA was extracted using the RNeasy Mini Kit (Qiagen).
The actual hybridization of cDNA to 3 “Mouse Genome 430 2.0 Array” (Affymetrix) of 3 chips per developmental stage, as well as normalization, was done in the laboratory of Prof. Norbert Hübner.

4. Results

To assess mechanosensory related traits of different modalities, as well as traits of temperature sensitivity that do not require mechanotransduction, a set of psychophysical and physiological testing procedures were established that are normally in clinical use for the measurement of sensory dysfunction.

To evaluate touch sensitivity, the vibration detection threshold (VDT) and the tactile acuity of the test persons were tested. For the VDT determination the CASE IV System was used (Gruener and Dyck 1994). A sinusoidal 125 Hz vibration was applied proximal to the nail of the little finger and the detection threshold determined as an amplitude. At 125 Hz both Meissner's corpuscles and Pacinian corpuscles respond. Tactile acuity was tested using the Tactile Acuity Cube (Van Boven and Johnson 1994), where the ability to detect the orientation of grids of different spacing with the fingertip of the little and index fingers was determined as a threshold in mm. Both tests are commonly used to monitor large diameter fiber function in the peripheral nervous system, often in the context of treatment of cases of diabetes mellitus.

Two aspects of hearing were examined in the test persons, hearing acuity and otoacoustic emissions. Hearing acuity is the psychophysical determination of the sound perception thresholds at a range of frequencies measured in decibels. During recording of click evoked otoacoustic emissions, the test person was passive and a pattern of clicks of different frequencies was applied to the ear. The recorded otoacoustic emissions generated by the outer cells were analyzed for the reproducibility of the evoked signal (Kemp, Bray et al. 1986) and also for the strength of the evoked signal itself (in dB), a parameter normally not considered in clinical evaluations.

Though not perceived consciously, the vascular baroreflex, in reaction to changes in blood pressure, can be measured by simultaneously recording the heart rate and the blood pressure at rest. Data was analyzed for baroreflex strength in the low (0.1 Hz) and high frequency (0.25 Hz) frequency bands by crosspectral analysis (deBoer, Karemaker et al. 1987) and also by the sequence technique (Bertinieri, di Rienzo et al. 1985). In addition to this, the number of detected baroreflex sequences over a certain period of time

(baroreflex sequence frequency) was analyzed, a parameter not usually considered in clinical evaluations.

As traits of the peripheral sensory nervous system that do not require mechanotransduction, four different temperature sensitivity traits were investigated using the TSA-II System (Shukla, Bhatia et al. 2005; Norbury, MacGregor et al. 2007). A thermal probe is placed on the volar forearm, and the ability to detect temperature changes to the cold and to the warm as well as heat and cold pain thresholds, were determined.

4.1 Age dependence of sensory traits

A cohort of 352 healthy individuals was tested, including the participants of the twin study described below, though not every individual performed every test. In this cohort, the age of participants ranged from 14 to 68 years. Besides the obvious deterioration of the visual or the auditory system correlated to age, the other sensory systems investigated in this study have also been shown to be age dependent (Meh and Denislic 1994; Stevens and Cruz 1996; Stevens and Choo 1998; Tank, Jordan et al. 2001; Stuart, Turman et al. 2003; Monahan 2007). To evaluate the effect of age on the variation in the investigated traits, the age dependence was analyzed in all healthy individuals tested.

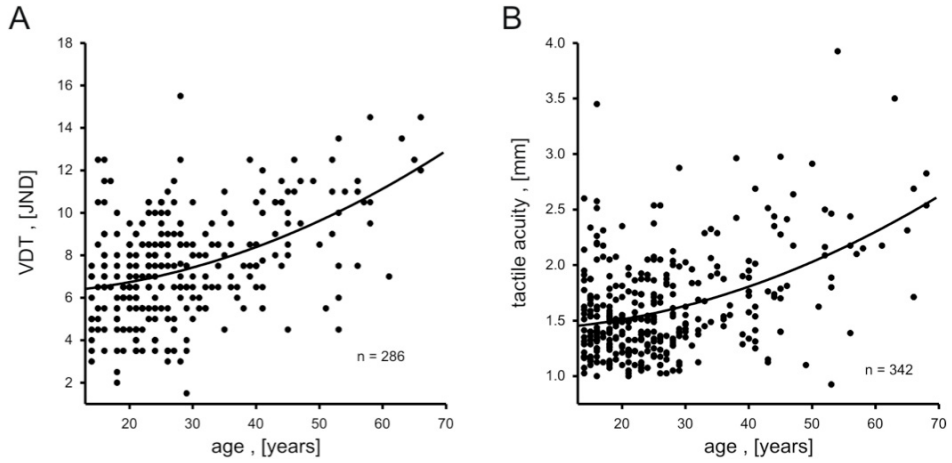

Figure 7: Age dependence of touch sensitivity traits. The vibration detection threshold (**A)** and the tactile acuity (**B)** showed strong age dependence, with a lower sensitivity in older subjects. Solid lines are regression lines. Equations of the regressions are listed in table 3.

The two touch sensitivity traits examined, vibration detection threshold and tactile acuity, showed strong age dependence (**Table 3**; **Figure 7**); poorer performances correlated with increasing age. Regression analysis shows that threshold values are around 2-fold higher in the oldest subjects of the cohort compared to the youngest. In both cases, the age dependence was best described by a polynomial function of the 2nd order.

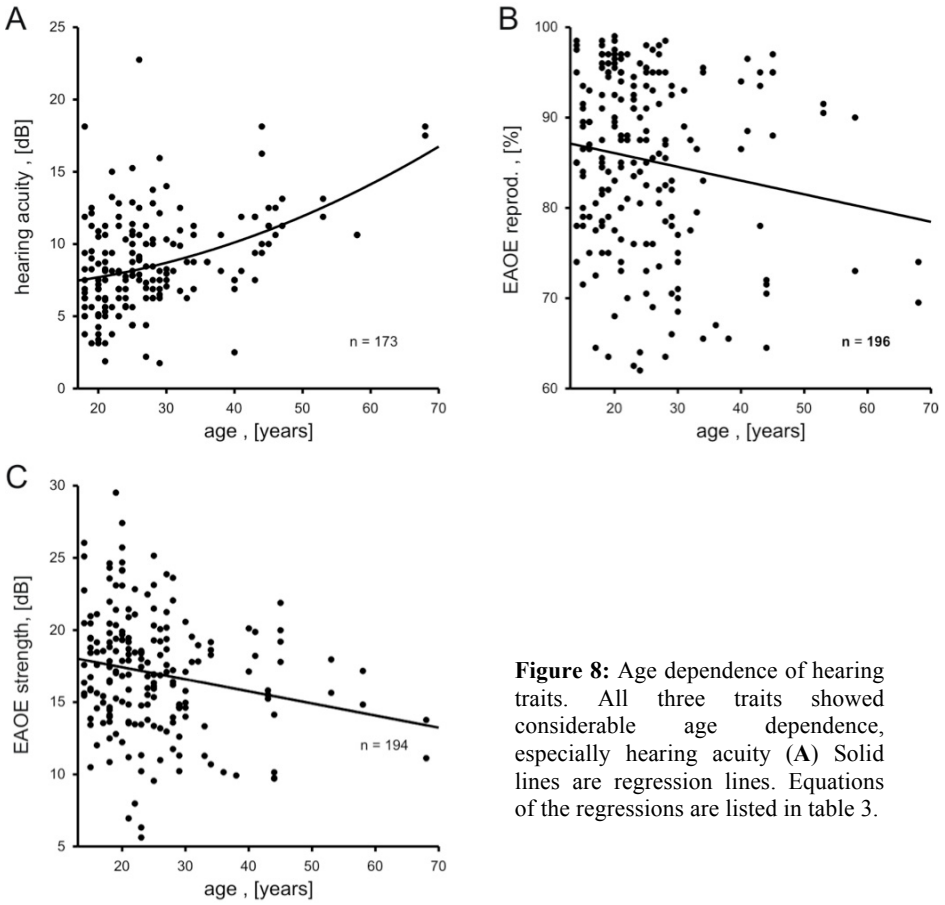

Figure 8: Age dependence of hearing traits. All three traits showed considerable age dependence, especially hearing acuity (**A**) Solid lines are regression lines. Equations of the regressions are listed in table 3.

The hearing traits also show a significant age dependence with better test results in the younger subjects (**Table 3**; **Figure 8**). The effect was strongest for the hearing acuity measured in decibels (**Figure 8A**). Here the regression shows that thresholds are around 100 % higher in the oldest subjects compared to the youngest ones. For hearing acuity a polynomial function of the 2nd order described the values better than a linear regression. For the two measures of the otoacoustic emissions the linear regression was preferred.

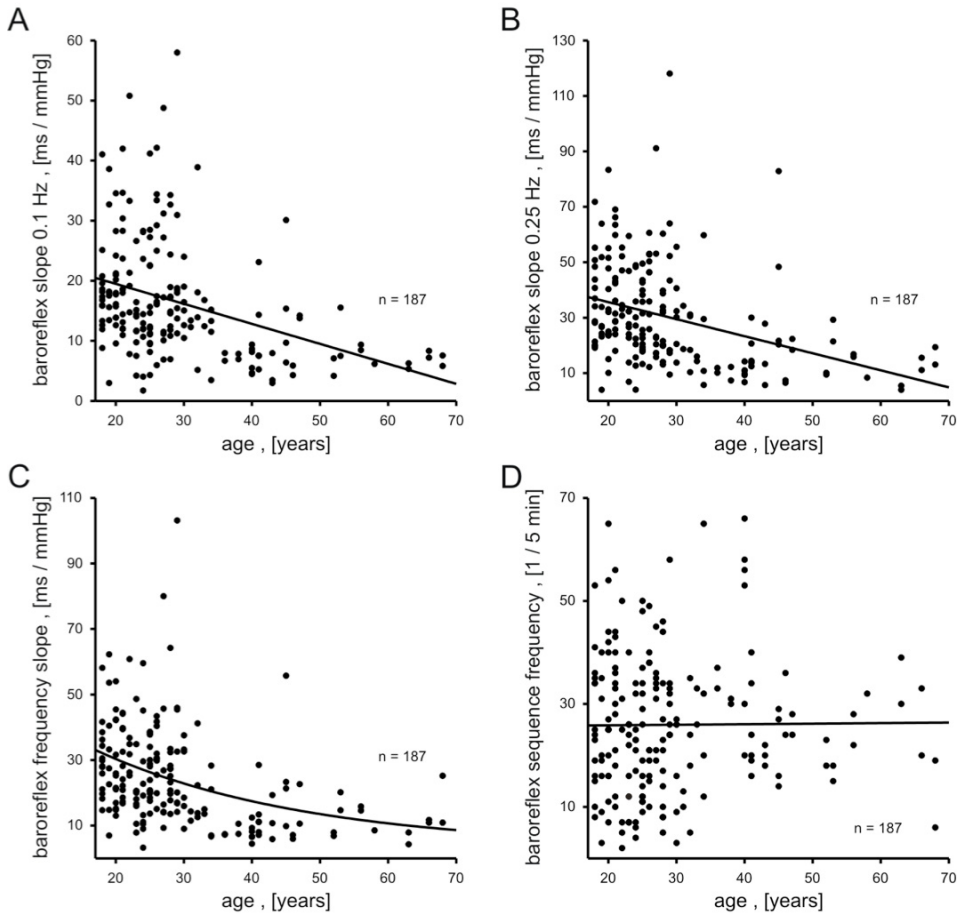

Figure 9: Age dependence of baroreflex traits. The high and low frequency baroreflex slopes as well as he baroreflex sequence slopes showed a strong age dependence (**A**,**B**,**C**) where the baroreflex frequency seems to be unaffected by age (**D**). Solid lines are regression lines. Equations of the regressions are listed in table 3.

The baroreflex slopes in both the high and low frequency bands, as well as the baroreflex sequence slope, showed strong age dependence (**Table 3**; **Figure 9A**,**B**,**C**); older subjects showed weaker reactions. The baroreflex sequence frequency does not seem to be affected by age and was indeed the only trait in this study that did not show a significant correlation between age and performance **(Figure 9 D)**.

Whereas the age dependence of the baroreflex slopes in the high and low frequency bands were best described by a linear regression, the change in baroreflex sequence slope was found to be better described by an exponential decay.

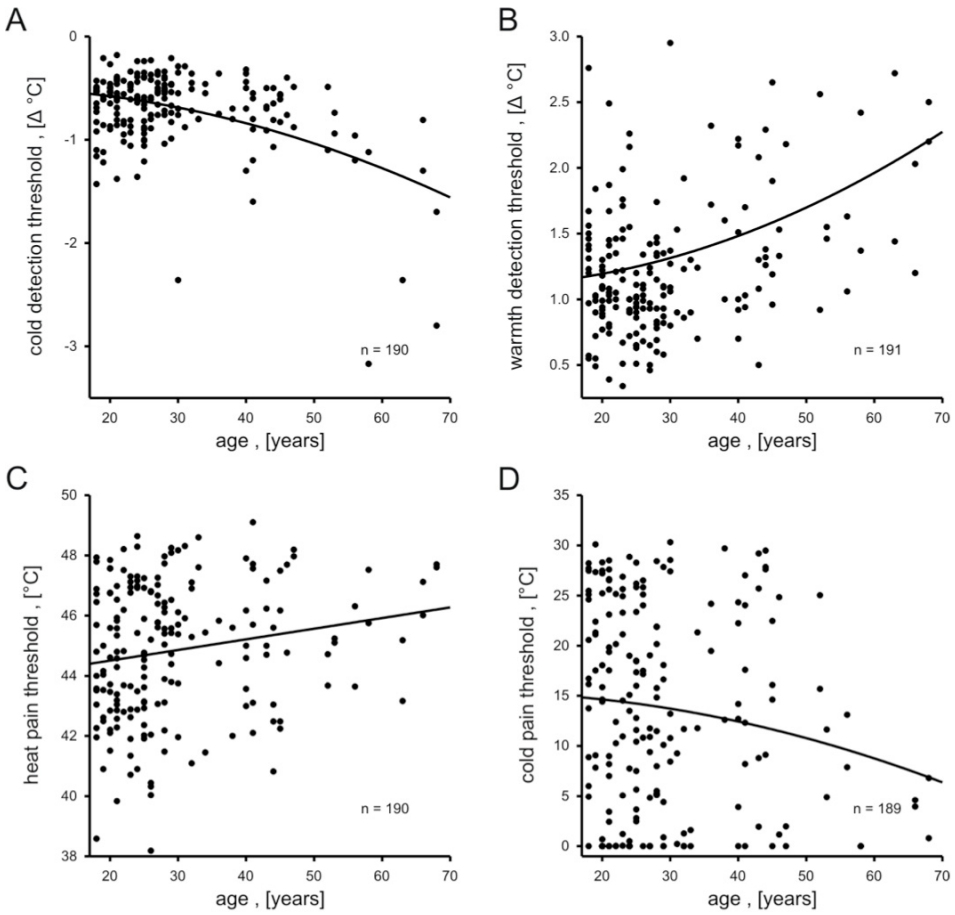

Figure 10: Age dependence of temperature sensitivity traits. All temperature traits showed age dependence. Solid lines are regression lines. Equations of the regression are listed in table 3.

Similarly, all of the four measured temperature sensitivity traits showed age dependence (**Table 3**; **Figure 10**). The cold and warmth detection thresholds were around 100 % higher in the oldest subjects compared to the youngest as can be seen from the regression lines. The age dependence of the cold, warmth and cold pain thresholds were best described by a polynomial function of the 2nd order, whereas for the heat pain threshold a linear regression was the better fit.

These results show a decrease in performance in all of the sensory systems investigated, which is very strong in most cases with values varying up to more than 100 % between different ages. The only exception is the baroreflex sequence frequency, which did not show a significant correlation between age and frequency values. Since the strong age dependence found in most traits could interfere with subsequent analysis we used the regressions shown in table 1 to correct the data for age or used age matched data.

Table 3: List of equations that describe the regressions for the age dependence of the sensory traits

Trait	Type of fit	Equation
Vibration detection threshold	Polynomial 2nd order	y [JND] = 6.19 + 0.00137 * age [years]^2
Tactile acuity	Polynomial 2nd order	y [mm] = 1.41 + 0.00025 * age [years]^2
Hearing acuity	Polynomial 2nd order	y [dB] = 6.89 + 0.00201 * age [years]^2
EAOE reproducibility	Linear	y [%] = 89.10 - 0.15205 * age [years]
EAOE strength	Linear	y [dB] = 19.10 - 0.08390 * age [years]
Baroreflex slope 0.1 Hz	Linear	y [ms / mmHg] = 26.14 - 0.33294 * age [years]
Baroreflex slope 0.25 Hz	Linear	y [ms / mmHg] = 47.92 - 0.61496 * age [years]
Baroreflex sequence slope	Exponential decay	y [ms / mmHg] = 51.68 * exp (-0.03231 * age [years]) + 3.246
Baroreflex sequence frequency	Linear	y [1 / 5 min] = 25.62 - 0.01067 * age [years]
Cold detection threshold	Polynomial 2nd order	y [°C] = 31.51 - 0.00022 * age [years]^2
Warmth detection threshold	Polynomial 2nd order	y [°C] = 33.10 + 0.00024 * age [years]^2
Heat pain threshold	Linear	y [°C] = 43.80 + 0.03531 * age [years]
Cold pain threshold	Polynomial 2nd order	y [°C] = 15.37 - 0.00184 * age [years]^2

4.2 Gender dependence of sensory traits

In our healthy cohort we examined a total of 143 males and 209 females. To evaluate the influence of gender on the variation of the traits studied here, the data of our cohort of healthy people was analyzed for gender differences. We noted that the cold detection threshold, warmth detection threshold, tactile acuity, EOAE reproducibility and strength as well as baroreflex sequence frequency showed a significant gender dependence in an age matched gender comparison **(Figure 11**). In all investigated traits women showed higher sensitivity or stronger responses than men, even though not all differences were significant.

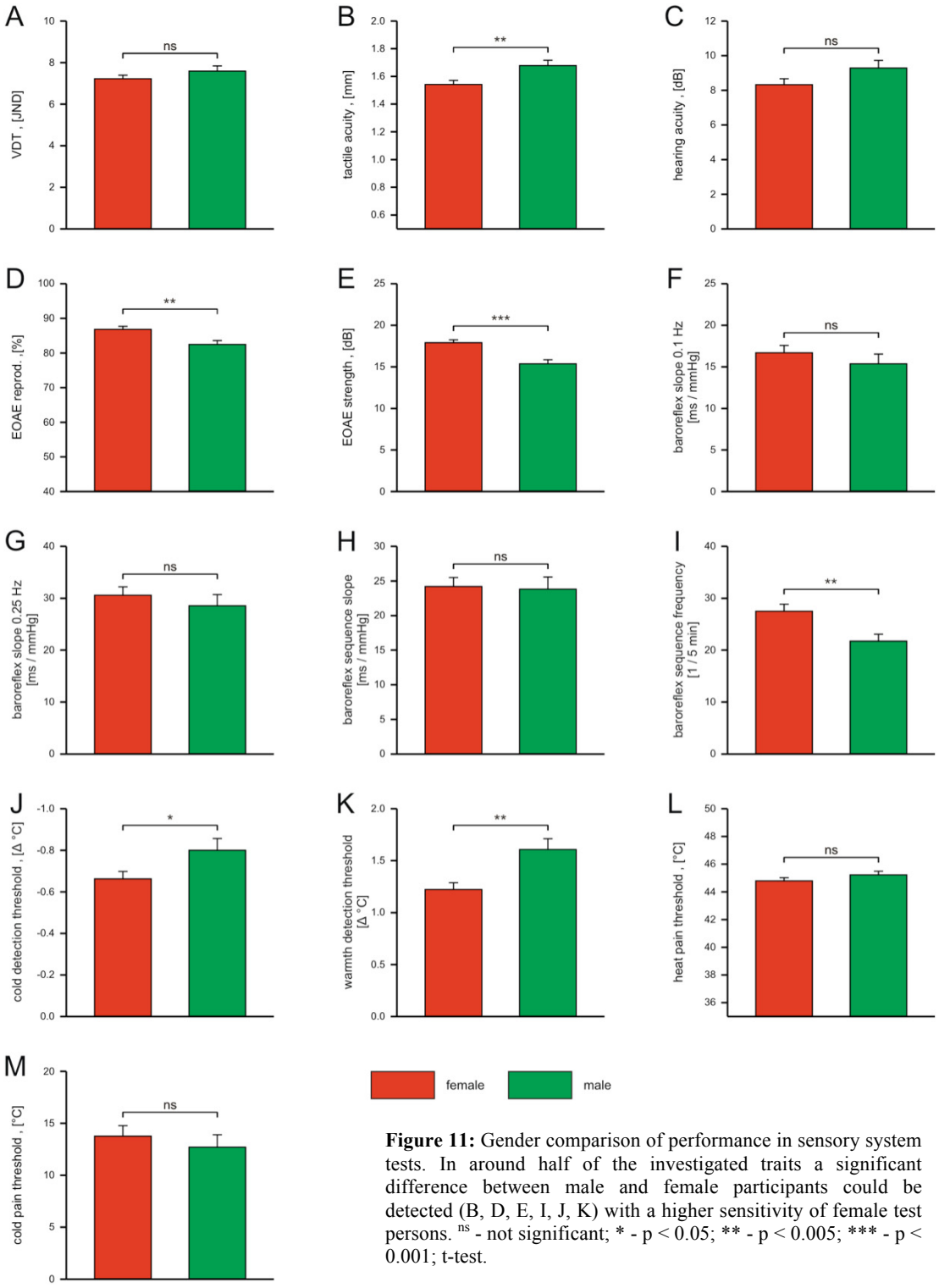

Figure 11: Gender comparison of performance in sensory system tests. In around half of the investigated traits a significant difference between male and female participants could be detected (B, D, E, I, J, K) with a higher sensitivity of female test persons. ^{ns} - not significant; * - $p < 0.05$; ** - $p < 0.005$; *** - $p < 0.001$; t-test.

4.3 Heritability of sensory traits

The procedures employed here to assess the different sensory systems are normally used to measure dysfunction. However, there is a high variability within a cohort of healthy persons. As shown above, some of this variability can be explained by age dependence and also, albeit to a lesser degree, by gender. Besides environmental influences, another parameter that could influence the variability of the tested traits is the stochastic genomic composition of the individuals. A measure of the influence of additive genetic effects is the heritability h^2, which was determined using a classical twin study. This has been done before for EOAE reproducibilty (McFadden and Loehlin 1995; McFadden, Loehlin et al. 1996), baroreflex slope (Tank, Jordan et al. 2001) and also for the heat pain threshold (Norbury, MacGregor et al. 2007) revealing a heritable component in all of these traits.
In this twin study the sensory traits were assessed in a total of 100 twin pairs. Of those 66 were monozygotic (MZ) and 34 dizygotic (DZ) twins. Zygosity was determined or confirmed by genotyping 11 satellite markers, the genotyping was done in the laboratory of Prof. Dr. Norbert Hübner at the MDC-Berlin.

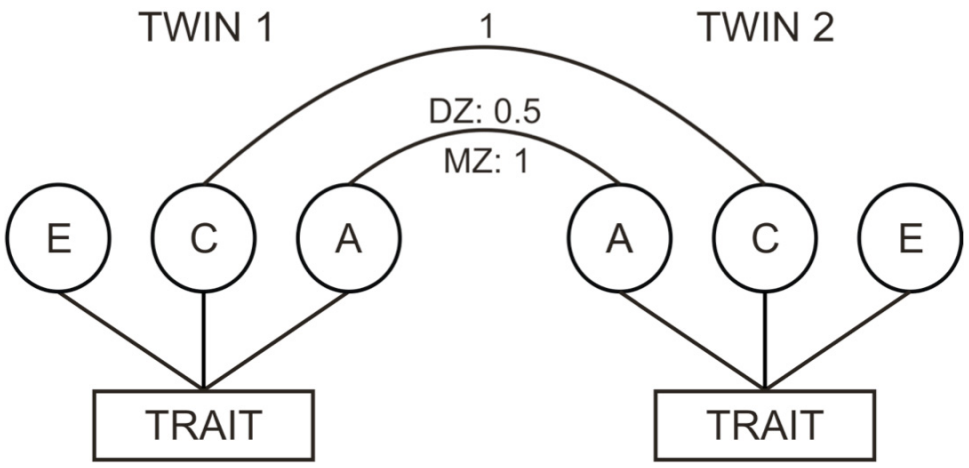

Figure 12: Model used to estimate the heritable component of trait variance. Influences on the variety of a trait are depicted by the straight lines. **E** is the unique environment component that influences a trait, which is unrelated in both twins. **C** is the common environment component, which is the same for both twins. **A** is the genetic component, which is the same for both twins if they are monozygotic (MZ) and half of it is the same if they are dizygotic (DZ). The influence of the **A** component to the trait variance corresponds to the heritability.

The power to estimate the heritability comes from comparison of the cross-twin correlations of the monozygotic and the dizygotic twin pairs. A cross-twin correlation that is higher in the MZ group than in the DZ group indicates a heritable component in the variability of invetigated traits.

A value for the heritabilities was estimated by performing structural equation modeling (Neale 2004). At first the data was fitted to a model where the phenotypic variance in the two groups, i.e. MZ and DZ twins, is determined by the influence of a additive genetic component (**A**), a common environment (**C**) and a unique environment component (**E**) (**Figure 12**). In this **ACE** model the influence of the **A** component to the trait variance corresponds to the heritability. In the next step, the model was tested again when either the **A** or the **C** component was removed from the model in order to see if the fit of the model improves, and the best fitting model, determined by the χ^2 value, was selected.

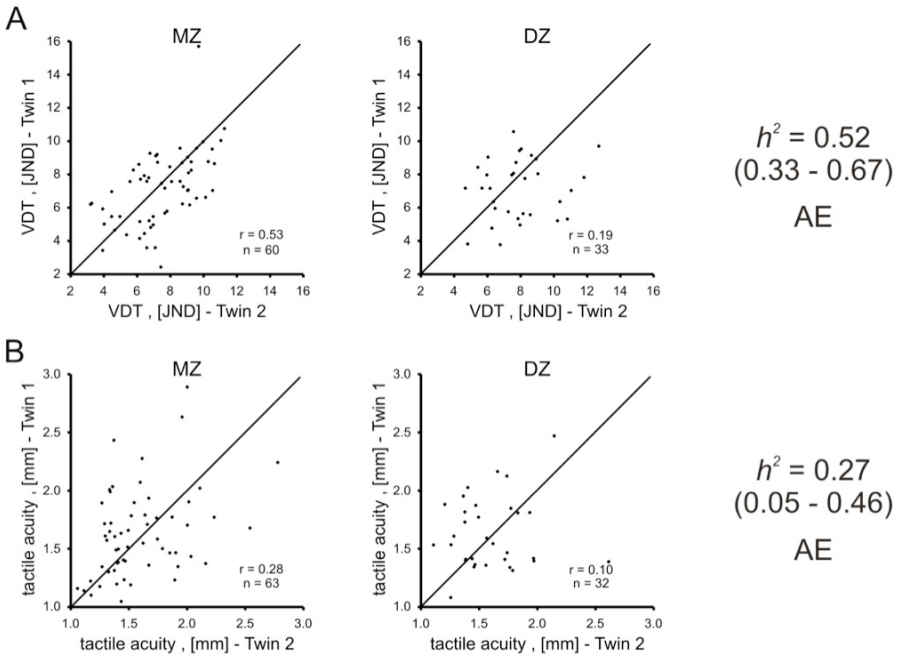

Figure 13: Cross-twin correlations and heritability estimates of touch sensitivity traits. For both vibration detection threshold and tactile acuity cross-twin correlations were higher in monozygotic (MZ) than in dizygotic twins (DZ) and significant heritability values could be estimated. r = intra-class correlation; h^2 = heritability estimate - 95 % confidence interval in brackets; AE = preferred model used to estimate heritability.

For both touch sensitivity traits, vibration detection threshold and tactile acuity, the cross-twin correlations were more than twice as strong in the monozygotic twin pairs than in the dizygotic twin pairs (**Figure 13**) with higher overall correlations for the vibration detection threshold. Significant heritability values could be estimated for both traits. The heritability estimate for tactile acuity was rather low at 0.27 (95 % CI = 0.05 - 0.46), whereas the estimate for heritability of vibration detection threshold was high at 0.52 (95 % CI = 0.33 – 0.67). For both traits the **AE** model was preferred and used to estimate the heritability.

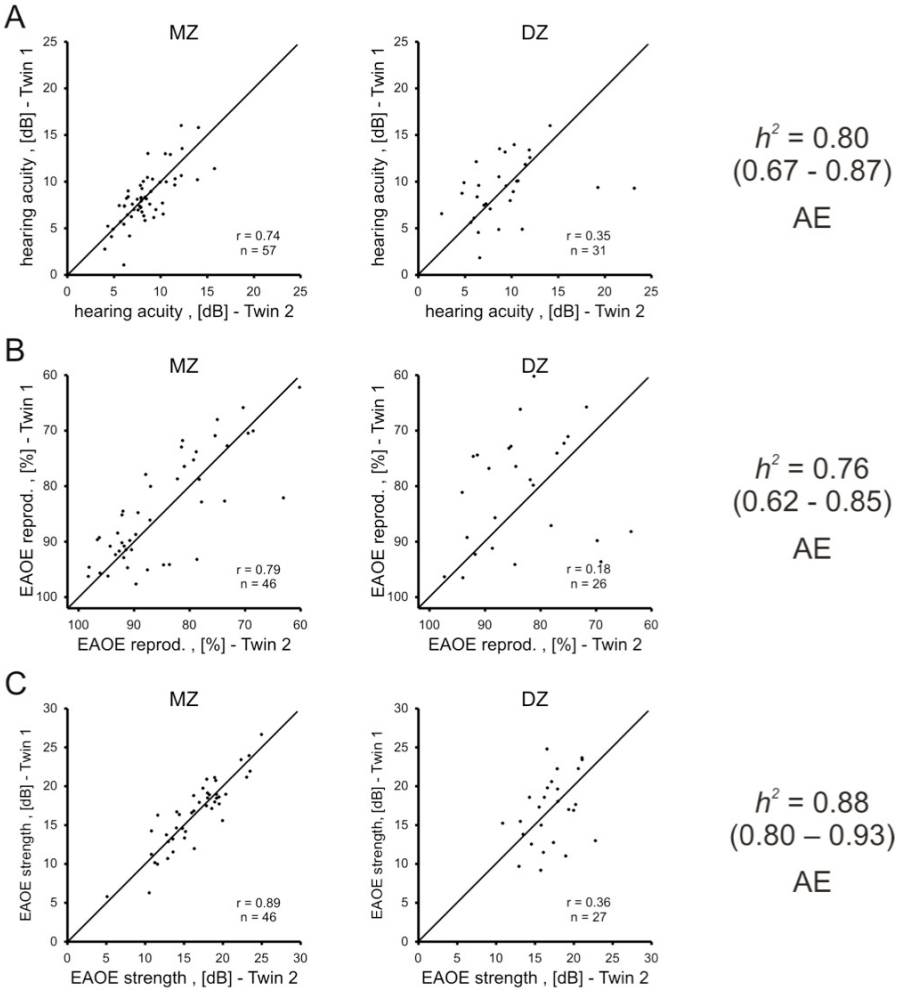

Figure 14: Cross-twin correlations and heritability estimates of hearing traits. For all three hearing traits the cross-twin correlations were much higher in monozygotic (MZ) than in dizygotic twins (DZ) and very high heritability values could be estimated. r = intra-class correlation; h^2 = heritability estimate - 95 % confidence interval in brackets; AE = preferred model used to estimate heritability.

For all three hearing traits the cross-twin correlations were more than twice as strong in the monozygotic twin pairs than in the dizygotic twin pairs with very high overall correlations (**Figure 14**).

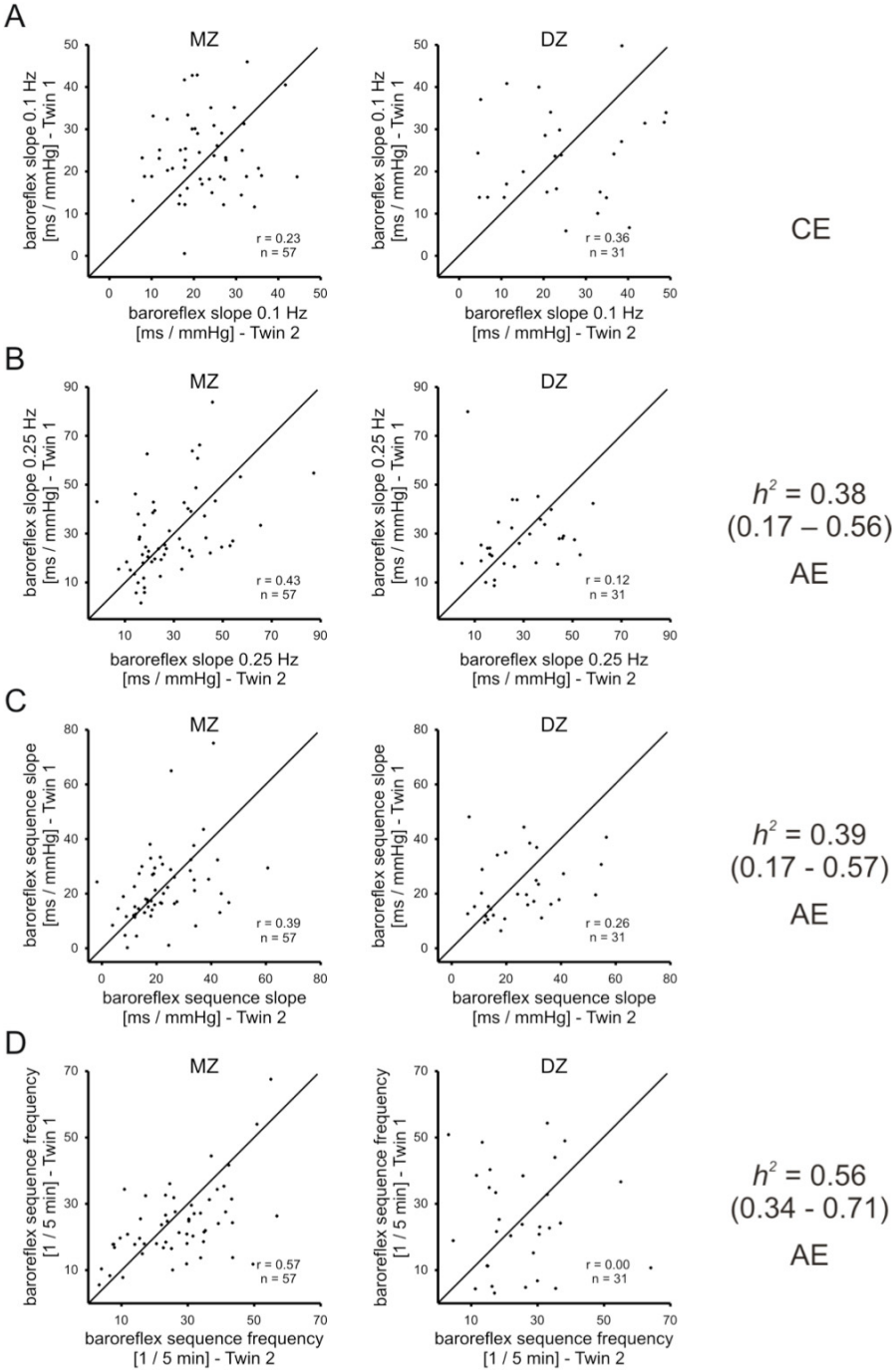

Figure 15: Cross-twin correlations and heritability estimates (where applicable) of baroreflex traits. For all baroreflex traits the cross-twin correlations were higher in monozygotic (MZ) than in dizygotic twins (DZ). Significant heritability estimates could be calculated for all traits except for the baroreflex slope at 0.1 Hz. r = intra-class correlation; h^2 = heritability estimate - 95 % confidence interval in brackets; AE / CE = preferred model used to estimate heritability.

The heritability estimates were exceptionally high, 0.80 (95 % CI = 0.67 – 0.87) for hearing acuity, 0.76 (95 % CI = 0.62 – 0.85) for EAOE reproducibility and even 0.88 (95 % CI = 0.80 – 0.93) for EAOE strength. For all three traits the **AE** model was preferred and used to estimate the heritability.

The cross-twin correlations for all baroreflex traits were higher in the monozygotic twin pairs than in the dizygotic twin pairs (**Figure 15**). Significant heritability estimates could be calculated for all traits, except the 0.1 Hz - baroreflex slope, using the **AE** model. The baroreflex slope at 0.25 Hz and the baroreflex sequence slope estimates were 0.38 (95 % CI = 0.17 – 0.56) and 0.39 (95 % CI = 0.17 – 0.57) respectively and higher for baroreflex sequence frequency at 0.56 (95 % CI = 0.34 – 0.71). A heritability value could not be calculated for the baroreflex slope at 0.1 Hz, because the best fitting model did not include a genetic component (**CE** model).

The cross-twin correlations for all temperature sensitivity traits, except the cold pain threshold, were higher in the monozygotic twin pairs than in the dizygotic twin pairs (**Figure 16**). Significant heritability estimates could be calculated for the cold and warmth detection thresholds using the **AE** model. The cold and warmth detection estimates were 0.40 (95 % CI = 0.16 – 0.60) and 0.37 (95 % CI = 0.14 – 0.56) respectively. A heritability value was not calculated for heat and cold pain thresholds because the best fitting model did not include a genetic component (**CE** model).

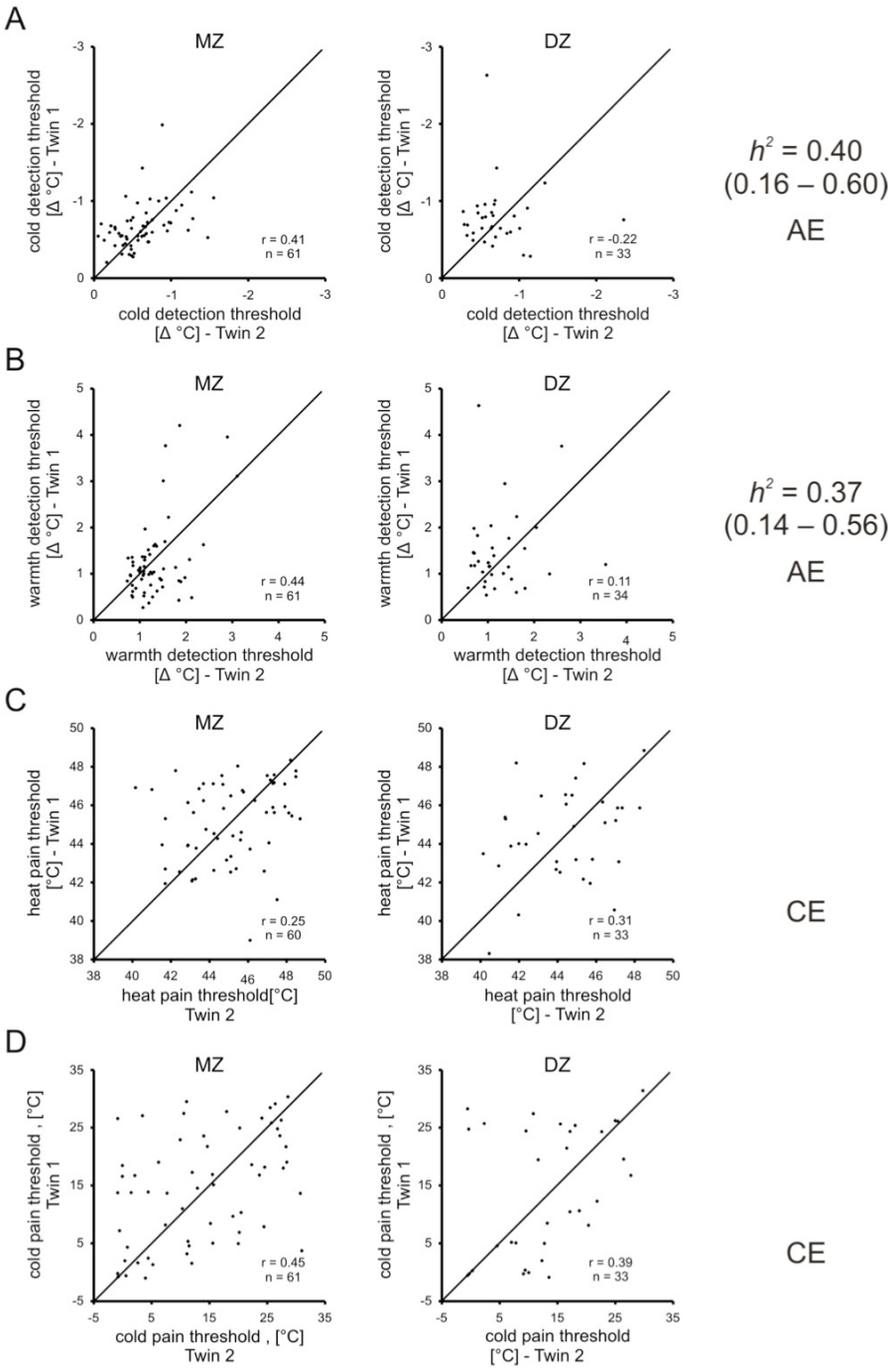

Figure 16: Cross-twin correlations and heritability estimates (where applicable) of temperature sensitivity traits. For all traits except the cold pain threshold the cross-twin correlations were higher in monozygotic (MZ) than in dizygotic twins (DZ). Significant heritability estimates could be calculated for warmth and cold detection thresholds. r = intra-class correlation; h^2 = heritability estimate - 95 % confidence interval in brackets; AE / CE = preferred model used to estimate heritability.

4.4 Cross-correlations between sensory traits

If we hypothesize a common genetic basis for different mechanosensory traits, we would expect to see a phenotypic correlation if we compare different traits in the same healthy individuals. This was done for the participants of the twin study described above, combined with an additional cohort of healthy individuals.

Table 5: Pearson correlations between the sensory traits. Intra-modal correlations highlighted in grey, mechano / temperature sensory correlations in yellow and mechanosensory correlations in red. ^{ns} = not significant; * = $p < 0.05$; ** = $p < 0.005$; *** = $p < 0.001$.

	Vibration detection threshold	Tactile acuity	Hearing acuity	EOAE reproducibility	EOAE strength	Baroreflex slope 0.1Hz	Baroreflex slope 0.25 Hz	Baroreflex sequence slope	Baroreflex sequence frequency	Cold detection threshold	Warmth detection threshold	Heat pain threshold	Cold pain threshold
Vibration detection threshold		0.21 ***	-0.01 ns	0.00 ns	-0.06 ns	0.04 ns	-0.05 ns	0.03 ns	-0.03 ns	0.02 ns	0.04 ns	0.07 ns	-0.03 ns
Tactile acuity			0.16 *	-0.16 *	-0.09 ns	0.07 ns	-0.01 ns	0.08 ns	-0.02 ns	-0.04 ns	0.08 ns	0.04 ns	-0.12 ns
Hearing acuity				-0.36 ***	-0.25 ***	0.17 *	0.03 ns	0.11 ns	-0.15 ns	-0.12 ns	0.16 *	-0.03 ns	0.06 ns
EOAE reproducibility					0.65 ***	0.06 ns	0.01 ns	0.02 ns	0.13 ns	0.08 ns	-0.05 ns	0.02 ns	-0.07 ns
EOAE strength						0.10 ns	-0.03 ns	-0.02 ns	0.22 *	0.04 ns	-0.07 ns	-0.04 ns	-0.03 ns
Baroreflex slope 0.1Hz							0.58 ***	0.71 ***	-0.13 ns	-0.12 ns	-0.04 ns	-0.07 ns	-0.05 ns
Baroreflex slope 0.25 Hz								0.77 ***	-0.22 **	-0.09 ns	-0.10 ns	-0.02 ns	0.02 ns
Baroreflex sequence slope									-0.22 **	-0.09 ns	-0.07 ns	-0.03 ns	-0.03 ns
Baroreflex sequence frequency										-0.04 ns	0.07 ns	-0.12 ns	0.02 ns
Cold detection threshold											-0.23 ***	-0.01 ns	0.04 ns
Warmth detection threshold												-0.02 ns	0.11 ns
Heat pain threshold													-0.60 ***
Cold pain threshold													

When we look at the Pearson correlations between the traits, we see that even the correlations between traits of the same modality reached only low values (**Table 5**; **Figure 17A**). For example, the correlation between vibration detection threshold and the tactile acuity was only r = 0.21. However, these correlations were highly significant with most p-values being less than 0.001. Significant correlations were also detected between traits of different mechanosensory modalities (**Figure 17B-E**; **Table 5**), i.e. between tactile acuity and hearing acuity with an r = 0.16 ($p < 0.05$) as well as EAOE reproducibility with an r = -0.16 ($p < 0.05$), between hearing acuity and baroreflex strength at 0.1 Hz with an r = 0.17 ($p < 0.05$) and between EOAE strength and baroreflex

sequence frequency. There was also one correlation between a mechanosensory and a temperature sensitivity trait, i.e. between hearing acuity and the warmth detection threshold with an $r = 0.16$ ($p < 0.05$).

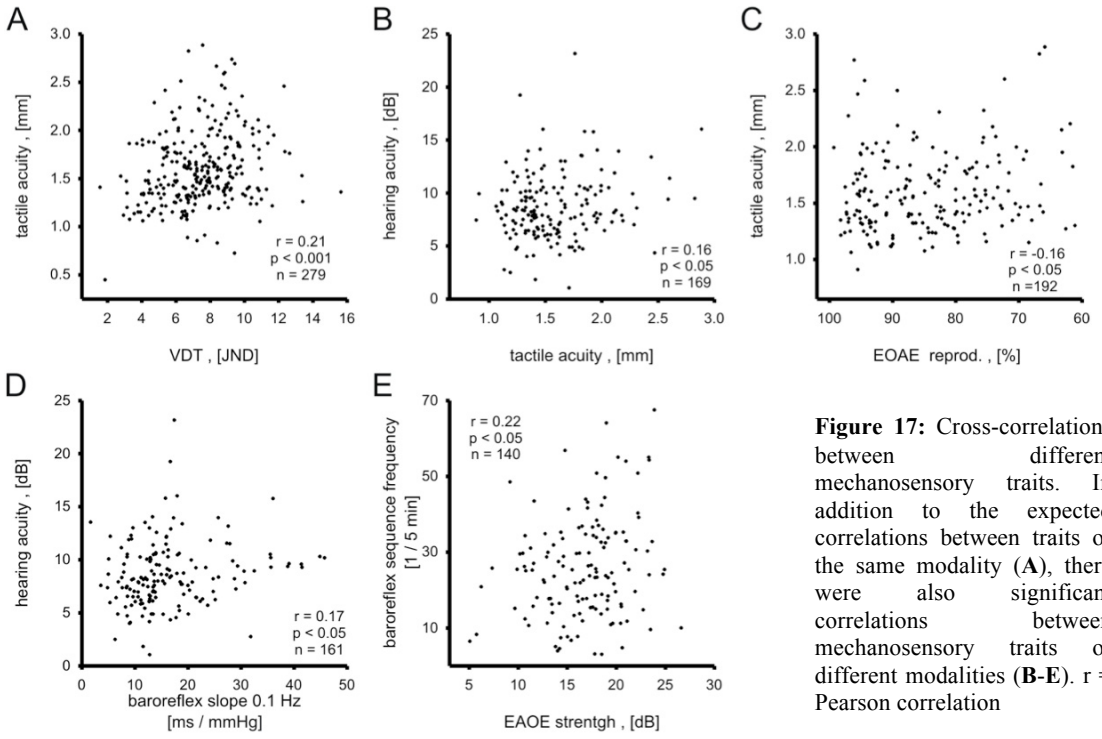

Figure 17: Cross-correlations between different mechanosensory traits. In addition to the expected correlations between traits of the same modality (**A**), there were also significant correlations between mechanosensory traits of different modalities (**B**-**E**). r = Pearson correlation

Since the observed relations are relatively weak it seems sensible to ask if the correlations remain if we account for the slight gender dependence observed in some traits, which can only partially be viewed as a genetic factor. If the gender-dependent traits are corrected for their gender differences (**Table 6**) only the correlation between tactile acuity and hearing acuity with $r = 0.15$ ($p < 0.05$), as well as between hearing acuity and baroreflex slope at 0.1 Hz with $r = 0.17$ ($p < 0.05$) remain significant. However, when the male and female participants were analyzed separately none of the above mentioned correlations reached statistical significance, which might be expected when sample sizes are reduced by half.

Table 6: Pearson correlations between the sensory traits with values corrected for gender differences where applicable. Intra-modal correlations highlighted in grey, mechano- / temperaturesensory correlations in yellow and mechanosensory correlations in red. ^{ns} = not significant; * = $p < 0.05$; ** = $p < 0.005$; *** = $p < 0.001$.

	Vibration detection threshold	Tactile acuity	Hearing acuity	EOAE reproducibility	EOAE strength	Baroreflex slope 0.1Hz	Baroreflex slope 0.25 Hz	Baroreflex sequence slope	Baroreflex sequence frequency	Cold detection threshold	Warmth detection threshold	Heat pain threshold	Cold pain threshold
Vibration detection threshold		0.21 ***	-0.01 ns	0.05 ns	0.01 ns	0.04 ns	-0.05 ns	0.03 ns	-0.01 ns	0.05 ns	0.00 ns	0.07 ns	-0.03 ns
Tactile acuity			0.15 *	-0.13 ns	-0.04 ns	0.07 ns	-0.02 ns	0.08 ns	0.03 ns	-0.01 ns	0.03 ns	0.02 ns	-0.11 ns
Hearing acuity				-0.35 ***	-0.23 **	0.17 *	0.03 ns	0.11 ns	-0.12 ns	-0.10 ns	0.14 ns	-0.03 ns	0.06 ns
EOAE reproducibility					0.62 ***	0.06 ns	0.02 ns	0.03 ns	0.06 ns	0.06 ns	0.01 ns	0.04 ns	-0.07 ns
EOAE strength						0.10 ns	-0.01 ns	-0.01 ns	0.13 ns	-0.02 ns	0.05 ns	0.00 ns	-0.03 ns
Baroreflex slope 0.1Hz							0.58 ***	0.71 ***	-0.13 ns	-0.13 ns	-0.04 ns	-0-07 ns	-0.04 ns
Baroreflex slope 0.25 Hz								0.77 ***	-0.23 **	-0.09 ns	-0.10 ns	-0.02 ns	0.02 ns
Baroreflex sequence slope									-0.21 **	-0.08 ns	-0.07 ns	-0.03 ns	-0.03 ns
Baroreflex sequence frequency										0.01 ns	0.10 ns	-0.12 ns	0.01 ns
Cold detection threshold											-0.21 **	0.01 ns	0.03 ns
Warmth detection threshold												-0.05 ns	0.13 ns
Heat pain threshold													-0.60 ***
Cold pain threshold													

4.5 Touch sensitivity and congenital hearing impairment

The next approach to test for a possible common genetic basis of touch and hearing was to test touch traits in individuals that suffer from a genetic hearing impairment. If a gene is affected in such an individual, and that gene is involved in both touch and hearing, one might be able to observe differences in touch sensitivity between controls and hearing impaired subjects. To do this we tested a cohort aged 14 – 20 years old that was recruited at a school for hearing impaired children; all subjects suffered from severe hearing impairment or hearing loss. We selected only students whose deafness or hearing impairment was congenital. It is estimated that in up to 70 % of cases of congenital hearing impairment, mutations in hearing related genes are causative for the pathogenic condition (Smith, Bale et al. 2005). In the remaining cases the hearing impairment is caused by developmental defects that are either not genetic, frequently induced by infection during pregnancy, or a consequence of medication, or the hearing impairment is

secondary to a genetic developmental defect. It is also sometimes unclear whether a hearing defect was acquired very early in life or if it is truly congenital.

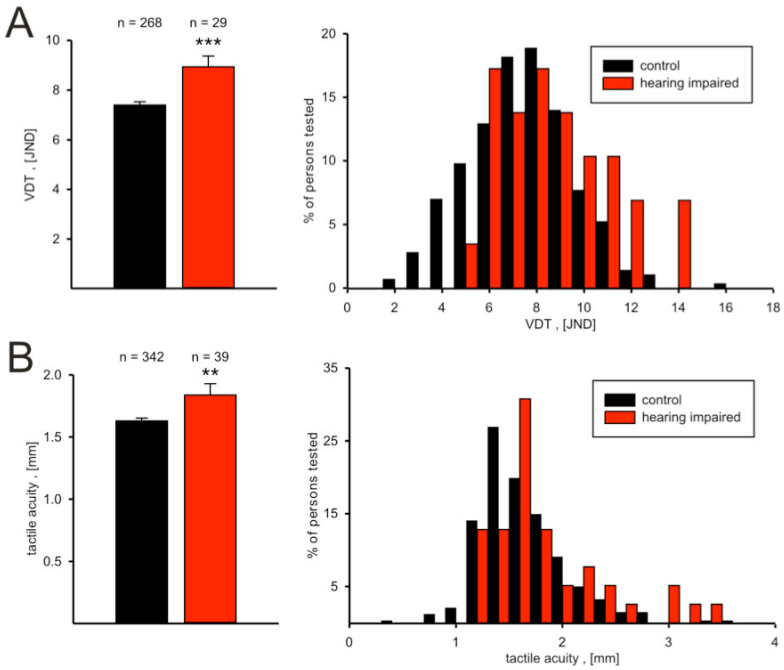

Figure 18: Touch sensitivity in a cohort of hearing impaired individuals compared to a cohort of normal hearing individuals. Mean performance in the mean vibration detection threshold test, as well as in the tactile acuity test, is lower in the hearing impaired cohort. ** = $p < 0.005$; *** = $p < 0.001$; t-test.

When vibration detection threshold and the tactile acuity mean values from the hearing impaired cohort were compared to those of a control cohort of normal hearing individuals, we observed that the mean performance in both touch tests is reduced in the hearing impaired cohort (**Figure 18**). The mean vibration detection threshold in the hearing impaired cohort was 8.93 ± 0.44 JNDs compared to 7.40 ± 0.13 JNDs ($p < 0.001$; t-test) in the age adjusted control cohort (corresponding to stimulus amplitudes of 2.23 µm and 3.23 µm, respectively). The mean tactile acuity was 1.84 ± 0.09 mm in the hearing impaired cohort comparing to 1.63 ± 0.02 mm in the control cohort ($p < 0.01$; t-test). In both tests the difference appeared to be caused by a subset of individuals with a poor performance, the values for these individuals lie at the right side of the normal distribution seen in the control cohort. It is important to note that these poor performing subgroups do not consist of the same individuals in both tests.

4.6 Touch sensitivity and the Usher syndrome

Genetic hearing impairment or deafness also occurs in a disease called Usher syndrome. In addition to hearing impairment, patients develop a condition that resembles retinitis pigmentosa. With the identification of the *Whirlin* gene there are nine different known genes, which cause three clinical subtypes of the Usher syndrome when mutated (Ebermann, Scholl et al. 2007). In the case of Usher syndrome type 1, the hearing loss is profound, the retinitis pigmentosa onset is in the first decade and vestibular dysfunction can also occur. In cases of Usher syndrome type 2, the hearing loss is comparatively mild and not all frequencies are equally affected and furthermore, retinitis pigmentosa onset can also be in the second decade. Usher type 3 is a rare form with progressive hearing loss and varying effects on vision and vestibular function. However, there are also mutations known in the Usher genes that cause nonsyndromic effects (Rivolta, Sweklo et al. 2000; Bork, Peters et al. 2001; Ahmed, Smith et al. 2002; Ahmed, Riazuddin et al. 2003; Mburu, Mustapha et al. 2003; Bolz, Bolz et al. 2004).

What makes the genes that are affected in Usher syndrome interesting in respect to a possible role in somatic mechanotransduction is that they are all expressed in the hair cell stereocilia (Kremer, van Wijk et al. 2006) and are therefore good candidates for being involved in the mechanotransduction process in hearing or have already been described to have such a role (Siemens, Kazmierczak et al. 2002; Ahmed, Goodyear et al. 2006).

A cohort of 65 Usher syndrome patients was tested for a possible alteration of touch sensitivity. The patients in this cohort were treated and counseled either in the Klinik für Audiologie und Phoniatrie of the Charité – Berlin or the Genetics Unit of the Hospital Unversitario, Valencia. For each of these patients a pathogenic mutation could be identified if it was not known already or the clinical subtype was determined by a clinical examination.

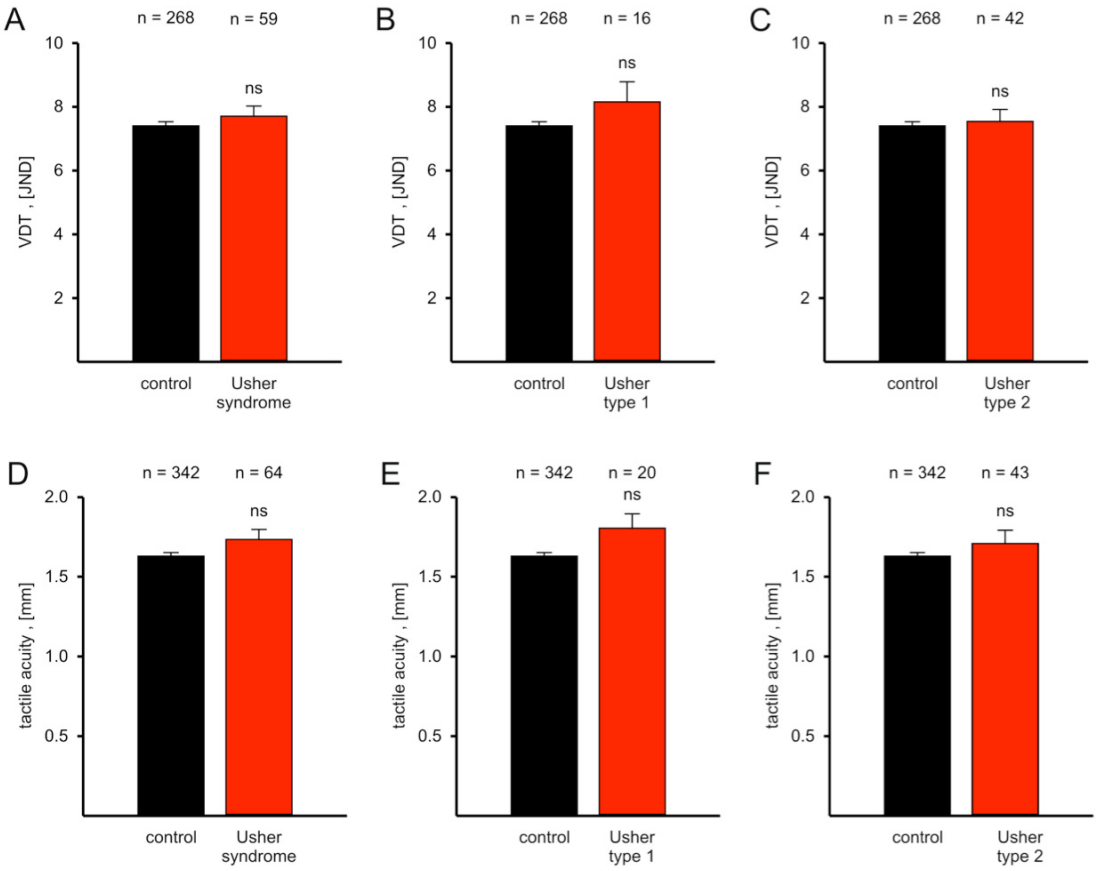

Figure 19: Touch sensitivity in cohort of people that suffer from the Usher syndrome. No significant difference in vibration detection threshold (**A–C**) or tactile acuity (**D-F**) could be detected when the control cohort is compared to the whole cohort of patients or to groups with the same clinical subtype. ns = not significant; t-test.

When the whole cohort of Usher patients was compared to a control cohort there was no significant difference in touch sensitivity (**Figure 19A**, **D**). The same was true when groups that had the same clinical subtype, Usher type 1 or Usher type 2, were compared to the control cohort (**Figure 19B**, **C**, **E**, **F**). In all cases there was a trend towards increased thresholds in Usher patients. To determine if greater differences, in more specified patient cohorts, underlie these trends the data was analyzed incorporating the genetic data.

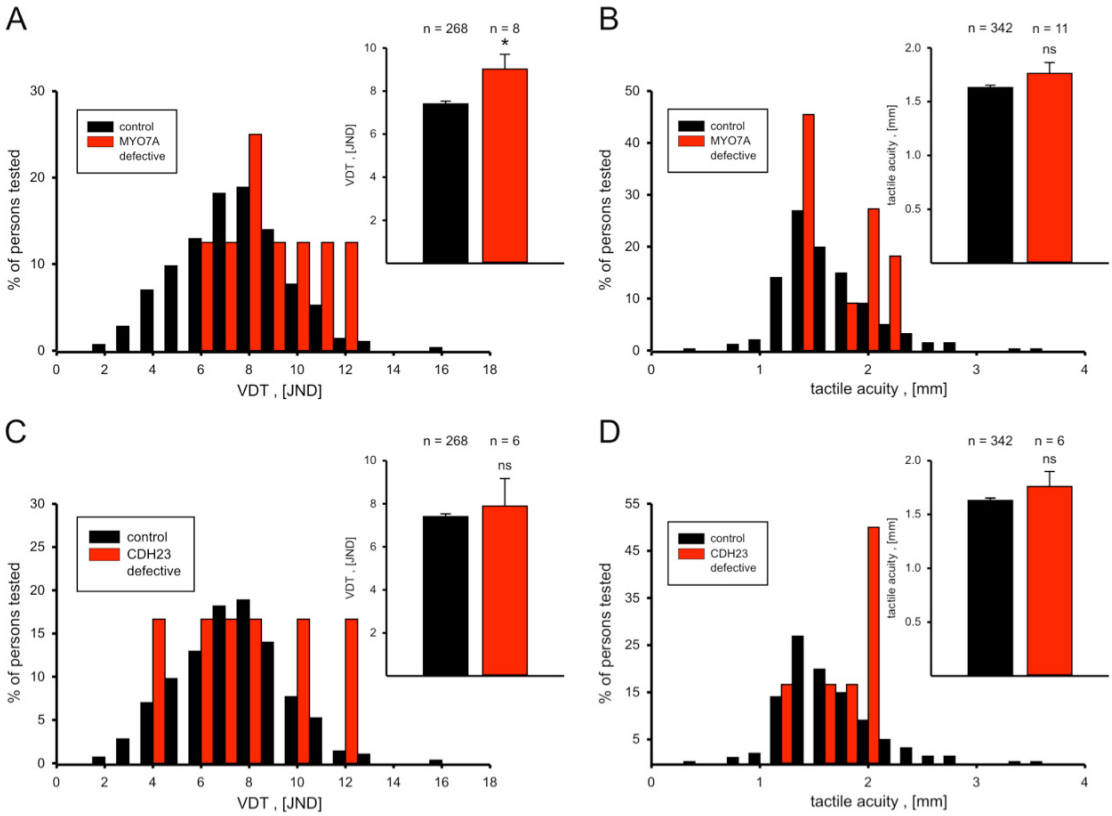

Figure 20: Touch sensitivity of patients suffering from the Usher syndrome type 1 according to their respective pathogenic mutations. The vibration detection threshold was significantly higher in patients with defective MYO7A (**A**) compared to a control cohort (the difference in tested individuals between the two tests resulted from inability of some test persons to follow the instructions for the vibration detection threshold test), whereas in the CDH23 defective group it is not (**C**). Tactile acuity was not significantly different in both groups (**B**, **D**). ^{ns} = not significant; * = $p < 0.05$; t-test.

Pathogenic mutations in two genes were present in the group of patients suffering from Usher syndrome type 1, the gene encoding the motor protein Myosin7a (MYO7A), which has shown to be involved in the adaptation of the hair cell transduction current (Kros, Marcotti et al. 2002), and *Cadherin23* (*CDH23*) whose gene product has been described as the tip link of the inner hair cell stereocilia, connecting the larger stereocilium to the tip of the adjacent smaller one (Siemens, Kazmierczak et al. 2002; Ahmed, Goodyear et al. 2006; Kazmierczak, Sakaguchi et al. 2007). The tips of the stereocilia are the proposed sites of the ion channel generating the transduction current (Beurg, Fettiplace et al. 2009). The vibration detection threshold in the group carrying pathogenic mutations in the *Myo7a* gene was significantly higher compared to an age adjusted control group (**Figure 20 A**). The mean threshold was 9.02 ± 0.68 JNDs compared to 7.40 ± 0.13 JNDs in the

control group ($p < 0.05$; t-test) (corresponding to metric values of 2.30 µm and 3.23 µm, respectively). Individual mutations and the corresponding thresholds are listed in **Table 7**. The dominant mutation listed in **Table 7** causes nonsyndromic hearing loss, which is not as severe as in other cases of Usher syndrome type 1 (Bolz, Bolz et al. 2004).

Table 7: Individual mutations in the *MYO7A* gene of the people tested for touch sensitivity and the corresponding vibration detection thresholds. *d* = dominant mutation

n	Mutation 1		Mutation 2		JND
3	c.2557C>T *d*	p.R853C *d*	-	-	9.33, 8.48, 10.77
1	c.470G>A	p.S157N	c.1454delT	p.L485RfsX14	10.13
1	c.2461C>T	p.Q821X	c.2461C>T	p.Q821X	7.53
1	c.6025delG	p.A2009PfsX32	c.6025delG	p.A2009PfsX32	6.35
1	c.3238A>T	p.K1080X	c.3508G>A	p.E1170K	12.12
1	c.640G>A	p.G214R	c.1342_1343delAG	p.S448LfsX2	7.43

In the group carrying mutations in *Cdh23* the threshold was not significantly enhanced (**Figure 20C**). The same was true for the tactile acuity in both groups (**Figure 20B, D**).

The majority of cases of Usher type 2 are caused by mutations in the gene *Ush2a*, which codes for a protein containing a transmembrane domain and large extracellular domain (Bhattacharya, Miller et al. 2002; van Wijk, Pennings et al. 2004). Interaction has been shown between USH2A and extracellular matrix proteins (Bhattacharya, Kalluri et al. 2004; Bhattacharya and Cosgrove 2005) as well as to other Usher proteins (Adato, Lefevre et al. 2005; Reiners, van Wijk et al. 2005; van Wijk, van der Zwaag et al. 2006), suggesting a role in connecting the extracellular matrix to the intracellular network of Usher proteins. Expression in the stereocilia has been reported to be restricted to the developing hair cells (van Wijk, Pennings et al. 2004; Adato, Lefevre et al. 2005; Liu, Bulgakov et al. 2007). In accordance with the high frequency of *Ush2a* mutations among Usher type 2 patients, of the 43 people in the cohort suffering from Usher syndrome type 2, 13 carried two identified pathogenic mutations in *Ush2a* and in 10 patients a pathogenic mutation was identified in one *Ush2a* allele. In the latter case it is likely that there is another unidentified mutation in the other *Ush2a* allele and that defective USH2A is responsible for the syndrome.

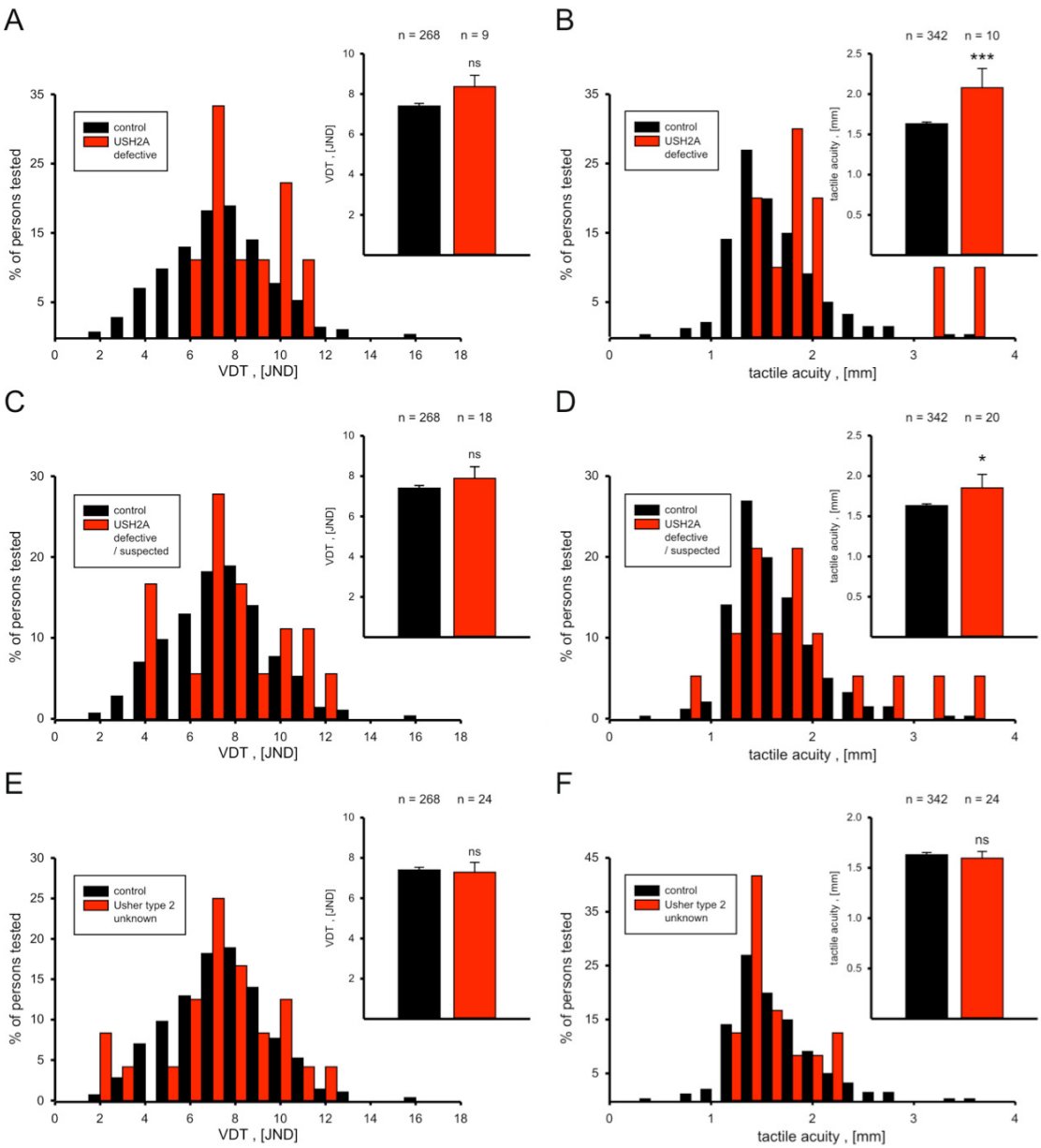

Figure 21: Touch sensitivity of patients suffering from the Usher syndrome type 2 according to their respective pathogenic mutations. Tactile acuity was significantly lower in patients with defective USH2A (**B**) compared to the control cohort, this was also true when individuals with only one identified mutation were added to the group (**D**).Vibration detection threshold is not significantly different in these groups (**A**, **C**). The group with clinically determined Usher type 2, with no identified mutation, showed normal mean values. ^{ns} = not significant; * = $p < 0.05$; *** = $p < 0.001$; t-test.

In the cohort carrying mutations in the *Ush2a* gene, tactile acuity was found to be significantly reduced. This was true when only those individuals were analyzed that have two identified pathogenic mutations (**Figure 21B**), here the mean acuity was 2.08 ± 0.24

mm compared to 1.63 ± 0.02 mm ($p < 0.001$; t-test) in the age adjusted control group, and also if those individuals were included that had only one identified mutation in the *Ush2a* gene (**Figure 21D**), here the mean acuity was 1.85 ± 0.17 mm compared to 1.63 ± 0.02 mm ($p < 0.05$; t-test) in the age adjusted control group. Vibration detection threshold was not significantly altered in the two USH2A cohorts (**Figure 21A**, **C**). Individual mutations and the corresponding thresholds are listed in **Table 8**.

Table 8: Individual mutations in the *Ush2a* gene of the people tested for touch sensitivity and the corresponding tactile acuity thresholds.

n	Mutation 1		Mutation 2		tactile acuity [mm]
1	c.2299delG	p.E767SfsX21	c.4381C>T	p.L16S	1.34
1	c.2299delG	p.E767SfsX21	c.11864G>A	p.W3955X	3.23
3	c.2299delG	p.E767SfsX21	c.2299delG	p.E767SfsX21	1.50, 1.80, 1.99
1	c.1724G>A	p.C575Y	c.11864G>A	p.W3955X	1.65
1	c.2135delC	p.S712X	c.2431_2432delAA	p.K811DfsX11	3.66
1	c.2431_2432delAA	p.K811DfsX11	c.2431_2432delAA	p.K811DfsX11	2.06
1	c.1214delA	p.N405IfsX3	c.4029T>A or G	p.N1343K	1.74
1	c.2299delG	p.E767SfsX21	c.11234dupA	p.Y3745X	1.81
3	c.2299delG	p.E767SfsX21	unknown	unknown	2.83, 2.37, 1.83
1	c.653T>A	p.Val218Glu	unknown	unknown	1.11
1	c.1606T>C	p.C536R	unknown	unknown	1.53
1	c.13316C>T	p.T4439I	unknown	unknown	1.36
2	c.11864G>A	p.W3955X	unknown	unknown	1.22, 0.80
1	c.486-14G>A	splice site	unknown	unknown	1.33

Interestingly, the touch sensitivity in the cohort of patients with a clinically diagnosed Usher syndrome type 2, in which the underlying mutation was unknown, was not different from the control group (**Figure 21E**, **F**).

4.7 Baroreflex function and the Usher syndrome

The baroreflex function is dependent on the detected change of mechanical force exerted on blood vessel walls, generated by changes in blood pressure. As shown in Chapter 4.4, there is a correlation between baroreflex function and hearing in healthy individuals on the phenotypic level. To test whether dysfunction of the auditory system might be

associated with an abnormal baroreflex function, as shown for touch sensitivity in the chapters above, baroreflex function was also measured in a cohort of individuals suffering from the Usher syndrome.

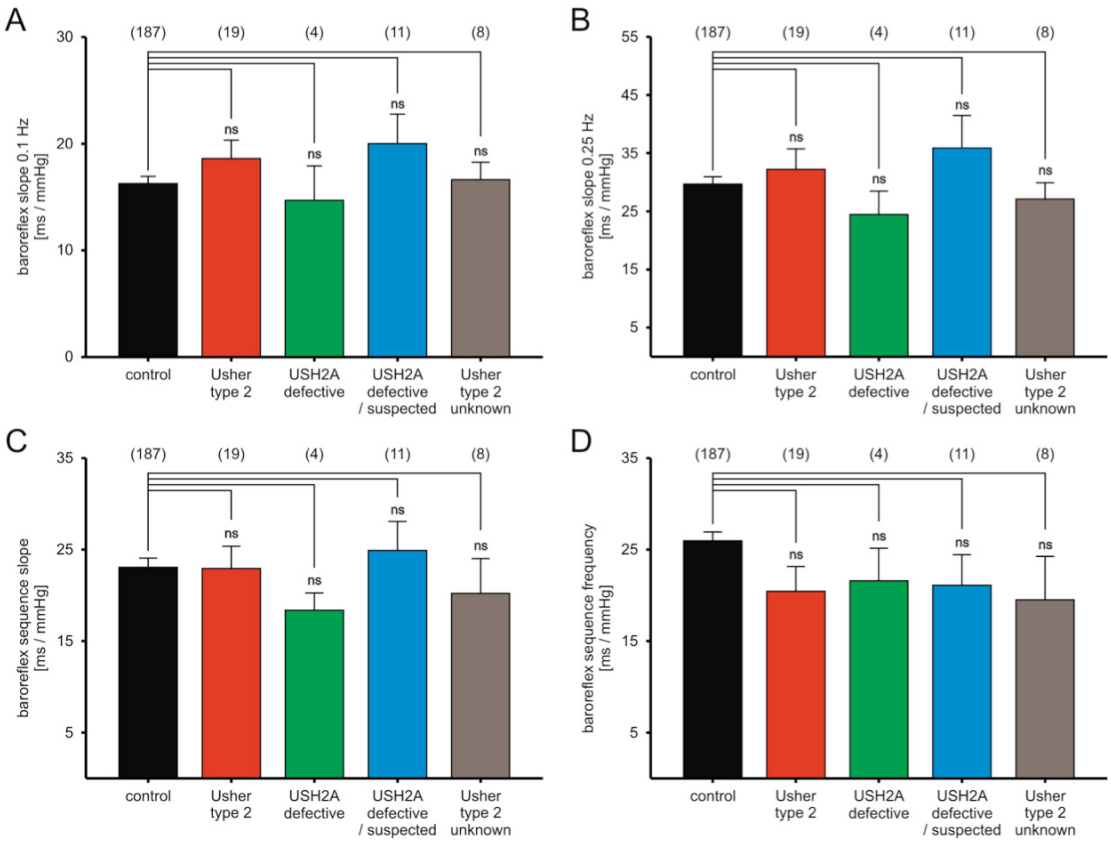

Figure 22: Four different measures of baroreflex function in a cohort of patients suffering from Usher syndrome type 2 in comparison to a control cohort. The cohort was also subdivided according to different genetic diagnoses. No significant difference in any of the different groups compared to the control cohort was measured. ^{ns} = not significant; t-test

All members of this cohort were diagnosed with Usher syndrome type 2 based on their clinical symptoms. None of the four investigated parameters, baroreflex slope at 0.1 Hz, baroreflex slope at 0.25 Hz, baroreflex sequence slope or baroreflex sequence frequency showed a significant difference from the age adjusted control group (**Figure 22A-D**). By analogy to the analysis of touch sensitivity in Usher patients, shown in **Figure 21,** one subgroup can be defined with two known pathogenic mutations in the *Ush2a* gene and one subgroup where additionally those individuals are included that have only one known mutation in the *Ush2a* gene. Whereas a reduced tactile acuity was observed for these

groups (**Figure 21B**, **D**), the measured baroreflex function was not significantly different (**Figure 22A-D**). The group of patients with no known mutation in *Ush2a* also showed no significant difference compared to controls.

4.8 Temperature sensitivity and the Usher syndrome

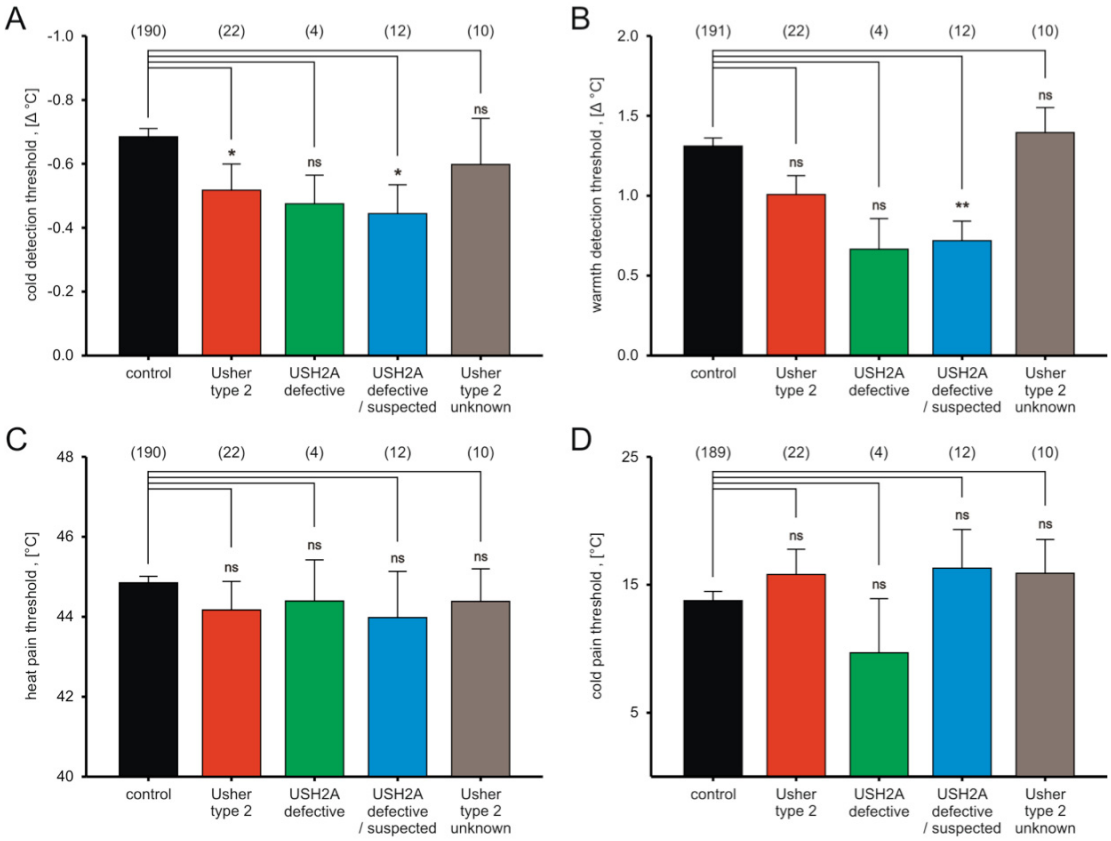

Figure 23: Four different measures of temperature sensitivity in a cohort of patients suffering from Usher syndrome type 2 in comparison to a control cohort. The cohort was also subdivided according to different genetic diagnoses. In the cold and warmth detection measurements there was a tendency for a higher sensitivity in all groups except the group with unknown underlying mutation when compared to a control group. This trend reached significance levels for the whole cohort in the cold detection test and for the group of patients with two pathogenic mutations in the *Ush2a* gene identified, combined with the group where only one such mutation has been identified in both tests. For heat and cold pain thresholds no significant difference was found. ^{ns} = not significant; * = $p < 0.05$; ** = $p < 0.01$; t-test.

Temperature sensitivity is dependent on the peripheral sensory system, but does not require the transduction of mechanical stimuli. To test if this system is affected in individuals suffering from the Usher syndrome we determined four temperature sensitivity parameters: cold detection threshold, warmth detection threshold, heat pain

threshold and cold pain threshold, as described above, in a cohort of individuals with a clinical diagnosis of Usher syndrome type 2.

In contrast to touch sensitivity, temperature sensitivity seems to be enhanced in people suffering from the Usher syndrome type 2. When the whole cohort is considered (**Figure 23A**), the cold detection threshold was significantly reduced with a mean of -0.52 ± 0.08 Δ °C compared to -0.68 ± 0.03 Δ °C in an age adjusted control cohort ($p < 0.05$; t-test) and there was a trend towards a reduced threshold in the warmth detection test. In the group of individuals with two identified *Ush2a* mutations there also was a trend towards reduced cold and warmth detection thresholds, but this group consisted of only four individuals. The difference reached significance levels when patients with only one identified *Ush2a* mutation were added **(Figure23A**, **B**). Here the mean cold detection threshold was -0.44 ± 0.09 Δ°C compared to -0.68 ± 0.03 Δ °C in the control group ($p < 0.05$; t-test) and the mean warmth detection threshold 0.72 ± 0.12 Δ °C compared to 1.31 ± 0.05 Δ °C in the control group ($p < 0.01$; t-test). Heat and cold pain thresholds did not differ from control levels.

4.9 Touch sensitivity and blindness

As seen in the previous chapter, sensory traits can be enhanced in a condition where one or in this case two senses are impaired. This has also been shown for touch sensitivity in blind people (Van Boven, Hamilton et al. 2000; Wan, Wood et al. 2010), although the reports are conflicting (Grant, Thiagarajah et al. 2000). To further investigate this, and to see how sensory deprivation other than hearing loss can influence performance in the specific touch sensitivity tests employed in this study, vibration detection thresholds and tactile acuity were measured in a cohort of blind people. The degree of blindness varied in the tested individuals, but was severe enough in all cases that the test persons were using the Braille system to read.

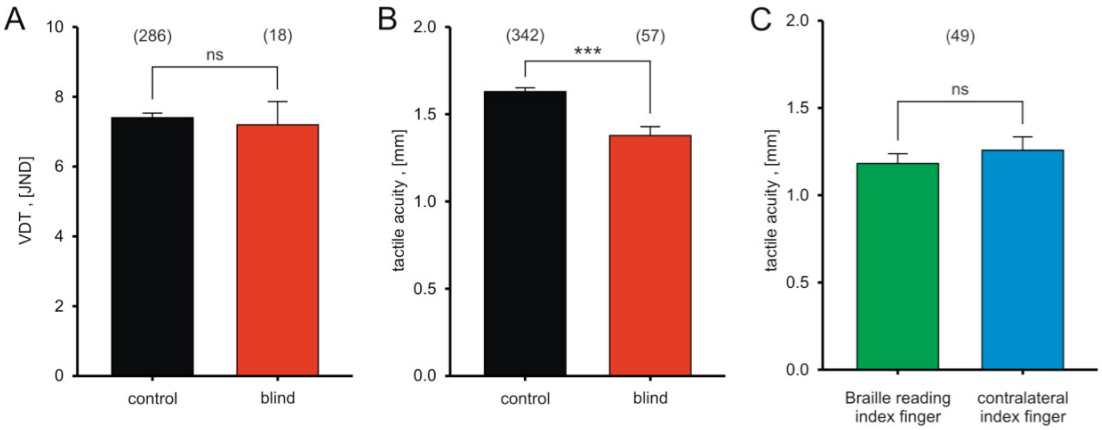

Figure 24: Touch sensitivity in a cohort of Braille reading blind people. The vibration detection threshold was not different in comparison to a control cohort, but the tactile acuity was significantly higher in the blind cohort. Tactile acuity measured on the Braille reading index finger and the contralateral index finger was not significantly different. ^{ns} = not significant; * = $p < 0.05$; *** = $p < 0.001$; t-test.

Whereas vibration detection threshold was not found to be different compared to the age adjusted control cohort (**Figure 24A**), mean tactile acuity was significantly better in the group of blind people (**Figure 24B**). Mean tactile acuity was 1.38 ± 0.05 mm compared to 1.63 ± 0.02 in the control cohort ($p < 0.001$). To examine how the use of fingers in Braille reading influences performance in tactile acuity tests, performance with the main Braille reading index finger was compared to the contralateral index finger, if the test person had a clearly preferred reading finger (**Figure 24C**). The mean acuity measured on the Braille reading finger was only a little higher and there was no significant difference.

4.10 Screening for genes involved in peripheral mechanotransduction in the developing mouse

4.10.1 Screening of candidate genes

At what time in development peripheral sensory neurons acquire their ability to transduce mechanical stimulations into a receptor current, and ultimately into action potentials, was not known until recently. The ability to record mechanically induced currents in acutely cultured peripheral sensory neurons (Hu and Lewin 2006) made it possible to investigate

transduction properties in developing embryonic sensory neurons. The time of onset of mechanosensitive currents in these neurons could be determined for different neuronal subtypes (Lechner, Frenzel et al. 2009). Around 70 % of large diameter (> 15 µM) mechanoreceptor neurons acquire mechanosensitivity at embryonic stage 13.5 (E13.5).
After E13.5 the proteins required for the transduction process are in place and the respective genes will be transcribed to maintain a functional sensory cell. If we now assume that genes that are exclusively needed for the mechanotransduction are not transcribed, or transcribed at a much lower level at the stages before E13.5, we can use this model to screen for mechanotransduction genes (**Figure 25**).

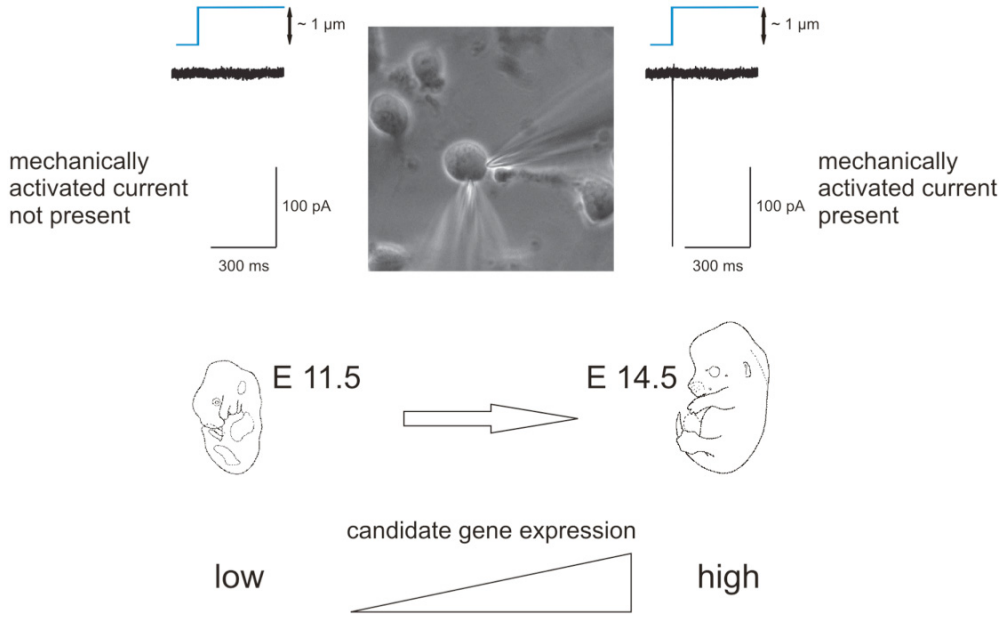

Figure 25: Scheme of the model used to screen for genes required for mechanotransduction in DRG neurons.

DRGs containing sensory neuron cell bodies were collected from mouse embryos of stage E 11.5, E 14.5 and of adult mice. RNA was extracted using a column based isolation method (RNeasy, Qiagen), and by real time PCRs the expression levels of genes between the different stages were compared for the genes of interest (see below).
To standardize the results they were normalized to the expression level of the transcripts of the housekeeping gene hypoxanthine guanine phosphoribosyl transferase 1 (*Hprt1*) because it was shown before to display relatively stable levels at different embryonic stages (Willems, Mateizel et al. 2006), albeit at earlier stages than in this study. To

confirm the eligibility of *Hprt1* as a standard, transcription of this gene was assessed in an initial experiment. The same amount of RNA was taken from three different preparations of each of the three developmental stages and reverse transcription and actual amplification reactions were then done side by side (**Figure 26**).

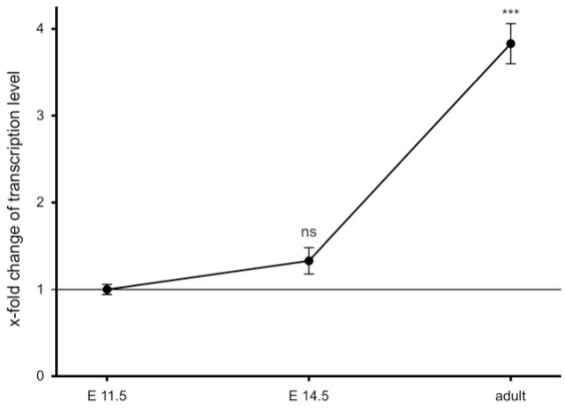

Figure 26: Relative change of *Hprt1* transcripts levels at embryonic stage E 14.5 and the adult mouse compared to stage E 11.5. Levels were not significantly higher at E 14.5, but were approximately 4-fold higher in the adult DRGs. ns = not significant; *** = $p < 0.001$.

Hprt1 appears to be only slightly regulated between stages E 11.5 and E 14.5 in our assay with a transcription level at E 14.5 that is around 1.3 times the value of E 11.5. Therefore, *Hprt1* was used for normalization when comparing transcript levels between stages E 11.5 and E 14.5. Compared to stage E 11.5, however, the transcription level at the adult stage is elevated around 3.8 times, which must be taken into account when data is analyzed.

At first, a set of 80 genes was tested that can be considered as candidates for being involved in mechanotransduction for one of the following reasons. Homologues of genes that have been shown to be involved in mechanotransduction in *C. elegans* (Bounoutas and Chalfie 2007) were included, these include ENaC- and ASIC-ion channels, as well as stomatin domain containing genes. Also included were all members of the TRP-channel family since several members of this family have been described to play a role in sensory processes (Lumpkin and Caterina 2007) and also show mechanosensitive properties (Lin and Corey 2005). Following the hypothesis that there might be common genes involved in touch and hearing, genes were also included that are known ”deafness genes“ and are expressed in the hair cells, the site of mechanotransduction in the ear.

Table 9: Expression profiles of 81 candidate genes in the developing DRG neuron as determined by real time PCR

	E 11.5			E 14.5			adult			x - fold change E 11.5 - E 14.5	t-test increase E 11.5 - E14.5
	mean	SEM	n	mean	SEM	n	mean	SEM	n		
Enacalpha	3.67E-03	7.11E-04	2	2.98E-03	2.65E-04	2	1.95E-03	3.78E-04	2	0.81	
Enacbeta	1.11E-03	9.18E-05	2	6.84E-05	1.25E-05	2	1.04E-05	0.00E+00	1	0.06	
Enacgamma	6.95E-03	3.83E-04	2	2.39E-04	1.66E-05	2	0.00E+00	0.00E+00	1	0.03	
Asic1	1.22E-01	1.05E-03	2	1.97E-01	2.47E-02	5	6.64E-02	5.97E-03	3	1.62	0.165
Asic1a	1.05E-01	2.58E-03	2	2.10E-01	1.27E-02	4	1.78E-02	4.14E-04	3	2.00	0.009
Asic1b	2.84E-02	3.83E-04	2	3.27E-02	4.55E-03	4	2.22E-02	1.41E-03	3	1.15	0.618
Asic2	2.10E-01	5.67E-03	2	1.40E-01	1.26E-02	5	2.41E-01	5.44E-03	3	0.66	
Asic2a	4.99E-02	1.65E-03	2	2.41E-02	3.86E-03	4	4.28E-02	8.45E-04	3	0.48	
Asic2b	3.54E-04	4.59E-05	2	1.02E-02	1.26E-03	4	1.60E-02	2.08E-03	3	28.85	0.011
Asic3	8.50E-03	3.54E-04	2	1.15E-01	1.15E-02	5	4.24E-01	5.98E-03	3	13.50	0.004
Asic4	1.38E-02	1.74E-03	2	2.05E-03	4.47E-04	5	1.98E-05	3.00E-06	3	0.15	
Trpc1	1.91E-01	2.36E-02	2	3.48E-01	2.88E-02	4	2.46E-01	1.19E-02	3	1.82	0.044
Trpc2	7.05E-05	1.76E-05	2	1.18E-05	4.95E-06	3	3.86E-06	2.82E-06	3	0.17	
Trpc3	1.36E-06	9.64E-07	2	7.20E-07	5.88E-07	3	4.02E-07	3.29E-07	3	0.53	
Trpc4	7.11E-04	4.09E-05	2	6.89E-03	1.22E-03	4	1.71E-03	8.78E-05	3	9.69	0.043
Trpc5	7.60E-03	1.06E-03	2	5.01E-03	4.44E-04	4	3.91E-03	1.09E-04	3	0.66	
Trpc6	1.12E-02	1.67E-03	2	2.66E-03	4.84E-04	4	2.95E-02	4.14E-03	3	0.24	
Trpc7	2.38E-04	3.14E-05	2	8.52E-04	1.74E-04	4	7.82E-04	1.37E-04	3	3.58	0.112
Trpm1	4.10E-05	5.22E-06	2	1.39E-04	1.79E-05	4	3.38E-05	5.78E-06	3	3.40	0.035
Trpm2	2.49E-02	1.43E-03	2	6.28E-02	4.87E-03	3	5.67E-02	5.81E-03	3	2.52	0.017
Trpm3	6.44E-03	7.88E-05	2	8.70E-02	7.90E-03	4	6.21E-02	4.51E-03	3	13.52	0.004
Trpm4	8.44E-02	1.01E-02	2	9.86E-02	9.92E-03	5	5.37E-02	2.53E-03	3	1.17	0.510
Trpm5	1.46E-03	5.13E-04	2	1.34E-02	2.16E-03	5	2.60E-04	3.94E-05	3	9.19	0.032
Trpm6	5.93E-03	5.20E-04	2	2.39E-03	3.05E-04	4	5.11E-03	3.26E-04	3	0.40	
Trpm7	2.16E-01	1.00E-02	2	9.29E-02	7.13E-03	4	3.05E-02	4.31E-04	3	0.43	
Trpm8	1.95E-06	1.38E-06	2	1.45E-04	7.93E-06	3	1.41E-02	2.06E-03	3	74.70	0.001
Trpv1	5.53E-04	4.07E-06	2	3.75E-01	5.19E-02	4	2.44E-01	5.23E-03	3	677.51	0.014
Trpv2	2.38E-01	9.31E-03	2	3.04E-01	4.61E-02	4	1.72E-01	1.13E-02	3	1.28	0.453
Trpv3	1.30E-03	5.56E-05	2	1.54E-03	2.64E-04	4	5.28E-04	5.08E-05	3	1.19	0.621
Trpv4	1.46E-02	4.10E-04	2	1.94E-02	2.19E-03	3	1.02E-02	6.13E-04	3	1.33	0.259
Trpv5	3.90E-05	2.10E-06	2	3.29E-05	6.32E-06	4	1.92E-05	3.70E-06	3	0.84	
Trpv6	1.84E-04	1.10E-04	2	1.15E-05	4.24E-06	4	3.27E-07	2.67E-07	3	0.06	
Trpml1	3.81E-01	3.30E-02	2	6.63E-01	1.43E-01	3	3.92E-01	1.82E-02	3	1.74	0.307
Trpml2	8.15E-06	5.76E-06	2	3.33E-07	2.72E-07	3	0.00E+00	0.00E+00	3	0.04	
Trpml3	1.14E-02	7.00E-05	2	6.13E-04	4.27E-05	3	2.54E-05	1.68E-06	3	0.05	
Trpa1	2.22E-03	4.45E-04	2	2.37E-03	3.67E-04	3	2.74E-01	3.82E-02	3	1.07	0.857
Pkd1	4.59E-02	3.47E-03	2	5.02E-02	1.87E-03	3	2.31E-02	1.60E-03	3	1.10	0.421
Pkd1l1	0.00E+00	0.00E+00	2	0.00E+00	0.00E+00	3	0.00E+00	0.00E+00	3	-	
Pkd1l2	9.12E-05	3.46E-06	2	8.05E-05	3.74E-06	3	8.56E-04	8.77E-05	3	0.88	
Pkd1l3	1.13E-03	1.25E-04	2	1.10E-04	1.12E-05	3	6.30E-05	9.66E-06	3	0.10	
Pkd2	5.55E-01	4.60E-02	2	3.10E-01	1.37E-02	3	9.55E-02	3.28E-03	3	0.56	
Pkd2l1	6.32E-03	6.31E-04	2	3.25E-03	3.03E-04	3	1.48E-04	3.58E-05	3	0.51	
Pkd2l2	1.81E-05	1.76E-06	2	4.27E-05	2.17E-05	3	4.60E-05	7.11E-06	3	2.36	0.525
Pkdrej	5.87E-04	3.87E-05	2	8.41E-04	5.90E-05	3	1.10E-03	8.58E-05	3	1.43	0.089

	E 11.5			E 14.5			adult			x - fold change E 11.5 - E 14.5	t-test increase E 11.5 - E14.5
	mean	SEM	n	mean	SEM	n	mean	SEM	n		
Stom	3.33E-03	2.48E-04	2	4.40E-04	1.14E-04	3	9.86E-04	2.79E-04	3	0.13	
Slp1	6.09E-02	3.35E-03	2	1.29E-01	7.83E-03	3	1.45E-01	6.70E-03	3	2.11	0.013
Slp2	3.66E-01	5.77E-02	2	1.56E-01	2.11E-02	3	5.99E-02	2.70E-03	3	0.43	
Slp3	1.44E-04	2.18E-05	4	5.47E-05	2.98E-05	3	1.87E-04	3.21E-06	3	0.38	
Sans	1.22E-03	1.14E-04	2	3.33E-04	5.13E-05	3	7.71E-03	1.64E-04	3	0.27	
Myo6	4.29E-01	7.36E-03	2	2.31E-01	1.24E-02	3	4.81E-02	1.97E-03	3	0.54	
Myh14	3.11E-02	7.63E-05	2	5.83E-02	5.23E-03	3	1.37E-01	8.98E-03	3	1.87	0.046
Myh9	1.71E+00	1.05E-02	2	9.09E-01	5.74E-02	3	6.27E-01	7.13E-02	3	0.53	
Myo1a	1.25E-02	7.34E-04	2	5.11E-02	3.25E-03	3	1.30E-01	6.74E-03	3	4.08	0.005
Actg1	1.19E+01	5.09E-01	2	7.21E+00	3.47E-01	3	1.85E+00	8.14E-02	3	0.61	
Tmc1	2.66E-05	3.67E-06	2	1.97E-06	8.50E-07	3	2.72E-05	7.20E-07	3	0.07	
Tmie	1.99E-03	4.03E-04	2	9.39E-04	6.88E-05	3	1.51E-03	1.39E-04	3	0.47	
Otof	1.63E-03	6.29E-04	2	4.10E-04	2.53E-05	3	3.64E-04	4.24E-05	3	0.25	
Ush2a	1.25E-03	1.71E-04	2	2.65E-03	5.25E-04	3	1.36E-04	7.85E-06	3	2.12	0.199
Gpr98	2.27E-02	6.45E-03	2	4.41E-03	5.86E-04	3	2.26E-04	2.28E-05	3	0.19	
Ush3a	9.42E-04	2.11E-04	2	1.24E-03	8.22E-05	3	1.23E-02	4.59E-04	3	1.31	0.336
Myo15	3.48E-05	1.87E-06	2	1.02E-05	5.11E-06	3	4.80E-06	5.10E-07	3	0.29	
Strc	6.08E-06	7.88E-07	2	2.47E-06	1.06E-06	3	1.62E-05	3.59E-07	3	0.41	
Myo3a	6.87E-05	1.77E-05	2	3.49E-05	3.64E-06	3	2.64E-05	4.16E-06	3	0.51	
Whrn	7.61E-03	7.05E-04	2	6.54E-03	1.25E-03	3	7.14E-03	2.10E-04	3	0.86	
Cdh23	5.60E-04	8.24E-05	2	7.80E-04	7.29E-05	4	1.42E-04	2.01E-05	3	1.39	0.203
Ush1c	2.12E-05	8.63E-06	2	4.49E-06	1.55E-06	4	2.50E-06	6.42E-07	3	0.21	
Myo7a	3.41E-04	1.22E-04	2	1.31E-04	5.81E-05	4	5.41E-05	9.43E-06	3	0.38	
Pres	4.40E-04	3.24E-06	2	6.33E-04	3.11E-05	4	3.20E-04	4.53E-05	3	1.44	0.023
Pcdh15	2.59E-02	5.77E-03	2	1.34E-02	3.08E-03	4	1.38E-03	1.08E-04	3	0.52	
Espn	6.70E-03	2.13E-03	2	1.36E-03	1.81E-04	4	5.41E-03	5.33E-04	3	0.20	
Kcnq4	2.23E-02	3.82E-04	2	1.90E-02	2.25E-03	4	2.72E-02	4.53E-03	3	0.85	
Pouf3	4.79E-01	3.39E-02	2	4.68E-01	7.89E-02	4	8.99E-02	4.09E-03	3	0.98	
Wfs1	2.75E-03	2.22E-04	2	2.02E-03	2.49E-04	4	9.16E-03	9.60E-04	3	0.74	
Cldn14	4.69E-03	2.18E-04	2	2.94E-03	5.54E-04	4	3.97E-03	4.62E-04	3	0.63	
Tflpc	6.93E-04	3.42E-04	2	8.34E-05	3.38E-05	4	1.79E-06	1.10E-07	3	0.12	
Triobp	2.35E-01	6.03E-03	2	1.27E-01	1.56E-02	4	2.43E-02	3.78E-03	3	0.54	
Tmhs	1.78E-02	7.61E-04	2	6.44E-02	9.80E-03	4	6.14E-02	3.88E-03	3	3.63	0.052
Diaph1	4.19E-01	3.27E-02	2	2.49E-01	2.71E-02	4	1.02E-01	4.49E-03	3	0.59	
Dfna5	4.05E-05	8.29E-06	2	4.86E-05	9.10E-06	4	1.80E-05	1.66E-06	3	1.20	0.664
Eya4	6.81E-02	7.37E-03	2	2.45E-02	2.31E-03	4	2.03E-03	7.32E-05	3	0.36	

Of the 80 genes tested (shown in **Table 9**), 12 showed an expression profile that corresponds to the emergence of mechanosensitivity in the DRG neurons. For 10 genes the measured increase in transcript level is significant and they are considered as positive hits in the screen for mechanotransduction genes (**Figure 27**). These genes displayed a more than two-fold upregulation between embryonic stages E 11.5 and E 14.5 as well as

a sustained high expression level at the adult stage. Five of these genes show a more than 10 fold upregulation (*Trpv1*, *Trpm8*, *Asic2b*, *Trpm3* and *Asic3*). The increase of transcript level observed for *Tmhs* (tetraspan membrane protein of hair cell stereocilia) and *Trpc7* was not significant.

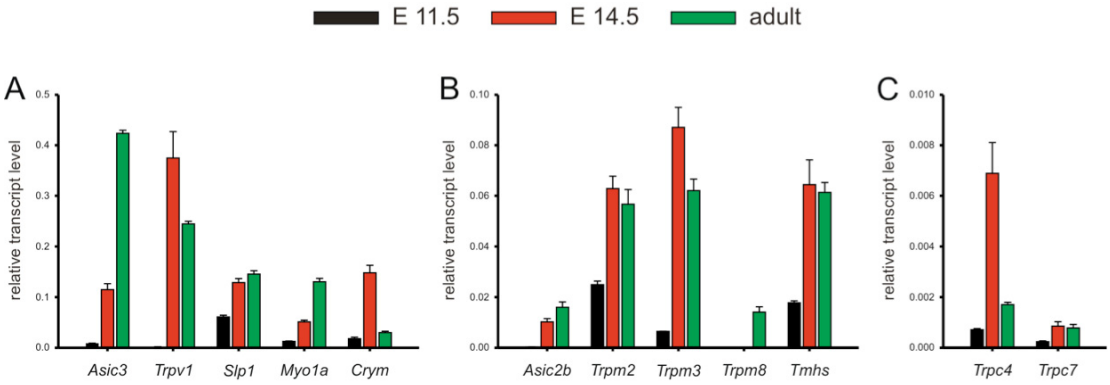

Figure 27: Expression profiles of candidate genes with a transcription profile that is in accordance with the acquisition of mechanotransduction properties in the developing mouse sensory neuron, i.e. the relative transcription level was upregulated more than 2-fold from embryonic stage E11.5 and E 14.5 with a sustained expression in the adult animal. All differences between E 11.5 and E14.5 stages, except for *Tmhs* and *Trpc7*, reached significance levels.

Acquisition of mechanotransduction can also be observed *in vitro*. Dr. Stefan Lechner could show that DRG neurons taken from stages where mechanosensitivity has not yet developed can develop mechanosensitivity when cultured under certain conditions (Lechner, Frenzel et al. 2009). Sensory neurons of acutely dissociated DRGs from E 12.5 embryos do not show mechanosensitivity (measured 4 h after cells were taken into culture). When these cells are cultured for another 20 hours in the presence of the neurotrophin NT-3, the majority of large diameter neurons have mechanosensitive currents. These cultures were used to test if the expression profiles of the genes that emerged from the previous screen are also consistent with the acquisition of mechanosensitive properties in this model. RNA was extracted from the respective cell cultures and real time PCRs were performed. For normalization the two neuronal marker genes neuron specific enolase (*Nse*) and microtubule-associated protein 2 (*Mtap2*) were used (**Figure 28**).

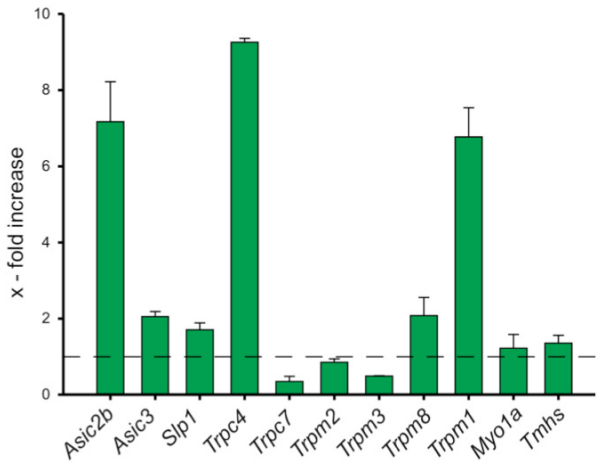

Figure 28: X – fold change of transcript levels between cultures of DRG neurons that were cultured for 4 h and 24 h in the presence of NT-3. Dashed line represents unchanged transcript level.

All genes except *Trpc7*, *Trpm2* and *Trpm3* were upregulated, the strongest upregulation was observed for *Asic2b*, *Trpc4* and *Trpv1* with 7.2 -, 9.3- and 6.8 – fold changes, respectively. These large changes in expression emphasized their status as candidates that emerge from this screen. However, we have to take into account the uncertainty concerning the normalization is bigger when compared, for example, to the normalization in the developing DRG. In culture the proportion of different cell types is changing dramatically during 20 hours and even though neuronal markers were used here there is no way of testing these markers with comparable amounts of neuronal RNA. Thus, the results of this experiment did not lead to an exclusion of candidates and the model was not applied in the screen described below.

Asic2b, *Asic3* and *Slp1* are homologues of genes involved in mechanotransduction in *C. elegans*. ASIC2b (amiloride-sensitive ion channel 2b) and ASIC3, members of the degenerin / epithelial sodium channel (DEG/ENaC) superfamily, have been described to have a role sensing mechanical stimuli, as well as protons, in mice (Price, Lewin et al. 2000; Price, McIlwrath et al. 2001). SLP1 (stomatin-like protein 1) is a membrane-bound protein containing a stomatin, prohibitin, flotillin, HflK/C- (SPFH-) domain (Mairhofer, Steiner et al. 2009). One further member of the family of stomatin-like proteins, SLP3, has been shown to be involved in mechanotransduction in mice (Wetzel, Hu et al. 2007) and Stomatin has been shown to modulate ASICs (Price, Thompson et al. 2004).

The ion channels TRPV1 (transient receptor potential, vanilloid subfamily, member 1) and TRPM8 are known to be functionally expressed in sensory neurons. TRPV1 is activated by protons, heat and capsaicin (Tominaga, Caterina et al. 1998) and TRPM8 by cold and menthol (McKemy, Neuhausser et al. 2002). The transcript levels of these channels in the developing DRG have previously been investigated and were shown to coincide with the emergence of their respective functional properties (Hjerling-Leffler, Alqatari et al. 2007). While emergence of *Trpm8* was reported to happen at the adult stage, in this screen we observed an upregulation at E 14.5 compared to E 11.5. However this upregulation was small and transcript levels in the adult DRG were also much higher (**Figure 27**).

Trpc4 was shown to be upregulated after nerve injury in DRG cells (Wu, Huang et al. 2008), among other functions suggested for TRPC4 is a role in endothelial barrier function (Cioffi, Lowe et al. 2009).

A role for TRPM2 or TRPM3 in sensory neurons has not yet been suggested. TRPM2 is activated by ADP-Ribose and reactive oxygen species (Fonfria, Marshall et al. 2004) and is thought to be involved in oxidative stress response in macrophages, T-lymphocytes and neutrophils (Sano, Inamura et al. 2001; Heiner, Eisfeld et al. 2003), but also in the process of insulin secretion in pancreatic β-cells (Togashi, Hara et al. 2006). TRPM3 is activated by pregnenolone-sulphate and seems also to be involved in pancreatic -cell function (Wagner, Loch et al. 2008).

MYO1A is expressed in the hair cells of the inner ear and has been shown to cause nonsyndromic sensorineural hearing loss when mutated (Donaudy, Ferrara et al. 2003), but its role in these cells is not yet understood. In fact MYO1A is also necessary for correct microvilli organization in the brush border of epithelial cells (Tyska, Mackey et al. 2005).

4.10.2 Genomewide screening

The screening described can be used not only for selected genes, but also for a genome-wide approach. To accomplish this, a "Mouse Genome 430 2.0 Array" by Affymetrix was used, RNA was extracted from DRGs from the same three developmental stages as

above and transcript levels assessed. Three chip arrays were carried out for one developmental stage with RNA from different preparations.
In order to get a reasonable number of positive hits in this screen, different, and more stringent, selection criteria were used than in the previous screen:

- the signal at the mechanoinsensitive stage E 11.5 must be absent, i.e. not higher than background noise levels
- a signal must be present at stage E 14.5 where the first cells show mechanosensitive currents
- there must be a further upregulation between stage E 14.5 and the adult stage as at this stage around 90 % of all DRG neurons show mechanosensitive currents (Lechner et al)
- at least one transmembrane domain must be present. Since the transduction complex is membrane-associated, this screen was focused on transmembrane proteins. Presence of transmembrane domains was determined by the previously described nature of the protein or when predicted transmembrane domains were listed in the Conserved Domains Database (CDD) by the NCBI

316 genes matched the required expression profile and of those, 83 genes contain one or more transmembrane domains. Five genes that have no described transmembrane domain were considered as candidates anyway because they are described to be membrane bound, to interact with candidate genes or to play role in sensory processes.
Next, the upregulation between stage E 11.5 and stage E 14.5 found for the respective genes was confirmed by realtime PCR in an experimental setup similar to the screen of the selected genes described above. A significant upregulation could be shown for the 52 genes that are listed in **Table 10**. Besides 15 genes of unknown function, there are some predominant functional groups. 11 code for transporters, ABC-transporters or associated proteins, 10 code for cell-adhesion proteins and six for proteins thought to have a structural function. The only gene that emerged from the screen of the selected candidates described previously and in this screen is the gene coding for the ion-channel TRPM2.

Table 10: Expression profile in DRG neurons determined by means of realtime PCR of those genes whose upregulation between E 11.5 and E 14.5 found in the microarray experiment could be confirmed.

	E 11.5		E 14.5		x - fold change E 11.5 - E 14.5	t-test increase E 11.5 - E 14.5	function
	mean	SEM	mean	SEM			
Abca8a	4.81E-04	2.88E-04	5.29E-02	2.06E-02	109.85	0.023	ABC-transporter associated
Ogn	8.30E-05	1.93E-05	1.13E-02	1.64E-03	136.47	0.021	matrix
1500015o10rik	2.26E-03	3.94E-04	1.73E-01	2.56E-02	76.83	0.022	unknown
Cdh1	1.87E-02	1.33E-02	9.18E-02	1.62E-02	4.92	0.010	cell-adhesion
Aqp4	1.54E-03	1.44E-04	2.83E-02	3.03E-03	18.34	0.013	water-channel
Ensmusg00000074335	5.62E-03	1.80E-03	8.40E-02	6.25E-03	14.94	0.007	unknown
Abhd3	4.75E-03	4.12E-05	7.23E-02	2.51E-03	15.22	0.001	enzyme
2900078e11rik	7.90E-03	1.92E-03	4.54E-02	6.19E-03	5.75	0.000	unknown
Mctp2	2.49E-03	3.89E-05	1.35E-01	1.82E-02	54.21	0.018	unknown
Mal	4.09E-03	1.18E-03	7.94E-02	7.75E-03	19.39	0.000	structural
Popcd3	2.17E-03	1.65E-04	2.93E-02	3.88E-03	13.46	0.020	unknown
Atp1a3	1.82E-01	3.25E-02	5.94E-01	5.03E-02	3.26	0.021	ABC-transporter
ItgblL1	9.67E-04	2.01E-05	1.41E-02	1.22E-04	14.54	0.000	cell-adhesion
Cntnap2	4.51E-03	3.35E-04	2.96E-02	3.07E-03	6.57	0.015	cell-adhesion
Nkain2	2.98E-02	2.99E-03	1.52E-01	2.64E-03	5.11	0.001	ABC-transporter associated
Ugt1a1	1.30E-02	2.24E-03	4.83E-02	2.84E-03	3.73	0.010	enzyme
Tmem16d	5.66E-02	2.16E-03	2.09E-01	1.66E-02	3.69	0.012	ion-channel
Fads6	8.42E-03	1.46E-05	4.42E-02	3.14E-03	5.25	0.008	enzyme
Susd2	6.29E-02	3.16E-03	2.77E-01	1.34E-02	4.40	0.004	structural
Adam23	2.24E-01	1.01E-02	7.21E-01	1.08E-01	3.22	0.044	cell-adhesion
Pcdha1	3.89E-01	8.61E-02	1.33E+00	8.51E-02	3.42	0.016	cell-adhesion
Pcdhb9	3.92E-02	6.12E-04	1.32E-01	4.78E-03	3.35	0.003	cell-adhesion
Ccdc109b	1.11E-02	1.70E-03	6.68E-02	5.20E-03	6.01	0.010	unknown
4732435n03rik	1.61E-04	1.36E-05	5.35E-04	1.85E-05	3.32	0.004	unknown
Slc41a2	5.14E-03	1.78E-05	1.89E-02	6.87E-04	3.68	0.002	transporter
Gramd1b	2.51E-03	6.53E-05	1.26E-02	1.60E-03	5.01	0.025	unknown
Bb146404	9.56E-03	4.31E-04	8.83E-02	2.91E-03	9.23	0.001	unknown
Lrrtm2	7.45E-02	5.16E-03	1.64E-01	1.67E-02	2.21	0.036	unknown
Opcml	1.01E-02	9.27E-04	3.26E-02	4.52E-04	3.22	0.002	cell-adhesion
Jakmip1	1.98E-02	1.50E-03	7.38E-02	5.61E-03	3.73	0.011	structural
Frmd3	4.98E-01	3.79E-02	1.38E+00	3.35E-02	2.77	0.003	structural
Hs3st5	4.77E-02	1.82E-03	1.51E-01	7.87E-03	3.17	0.006	enzyme
Tln2	1.48E-01	2.04E-02	4.07E-01	1.62E-02	2.75	0.010	structural
SlcC24a2	1.59E-02	9.08E-04	4.19E-02	5.92E-03	2.64	0.049	transporter
Abca8b	1.48E-04	1.96E-05	2.00E-03	2.01E-04	13.57	0.012	ABC-transporter
Lsamp	1.14E-01	1.39E-03	3.75E-01	3.43E-02	3.27	0.017	cell-adhesion
Cd55	6.61E-02	1.36E-02	1.47E-01	5.10E-03	2.23	0.030	immune response
Pcnxl2	6.77E-03	1.13E-03	3.87E-02	3.81E-03	5.72	0.015	unknown
Trpm2	3.03E-02	3.14E-03	6.11E-02	6.33E-03	2.01	0.049	ion-channel
Bean	7.25E-02	6.81E-03	1.23E-01	1.53E-02	1.70	0.039	unknown
Bc062109	3.88E-02	1.09E-03	1.07E-01	2.02E-02	2.77	0.020	unknown
Slc7a4	6.52E-02	1.08E-02	1.96E-01	3.03E-02	3.00	0.016	transporter
Bb181834	1.08E-04	2.12E-05	1.91E-03	2.31E-04	17.78	0.002	unknown
Slc10a6	5.55E-04	1.60E-04	2.27E-02	2.74E-03	40.93	0.002	transporter
Cldn1	1.46E-02	7.25E-04	2.40E-02	2.16E-03	1.64	0.015	cell-adhesion
LgalS3bp	9.46E-03	1.62E-03	3.83E-02	7.92E-03	4.05	0.019	immune response
Slc7a2	1.24E-02	7.37E-04	2.05E-02	1.17E-03	1.64	0.008	transporter
Lrrc3	6.72E-02	2.53E-03	1.24E-01	3.01E-03	1.85	0.001	unknown
Slc16a9	8.52E-03	1.38E-03	3.47E-02	9.21E-03	4.07	0.035	transporter
Itgb4	1.07E-02	2.99E-03	6.40E-02	3.21E-03	6.01	0.001	cell-adhesion
Npal2	6.58E-03	7.53E-04	1.62E-02	3.46E-03	2.47	0.039	nuclear protein
Fxyd2	2.73E-02	7.15E-03	8.60E-02	1.99E-02	3.15	0.044	ABC-transporter associated

5. Discussion

In this study we have investigated the genetics of different mechanosensory traits and the hypothesis that there are common genetic factors involved in different mechanosensory systems. We specifically investigated if there might be common genes involved in both touch and hearing. To address these questions, psychophysical and physiological tests were performed in different cohorts. In a classical twin study we could show a heritable component in touch sensitivity, hearing and baroreflex sensitivity. Studying cohorts suffering from different forms of congenital hearing loss, a reduced touch sensitivity related to congenital hearing loss was revealed and specifically in patients that suffer from the Usher syndrome caused by mutations in the genes *Myo7a* and *Ush2a*. In contrast, a cohort of blind people showed a higher tactile acuity.

5.1 Age dependence of sensory traits

Altogether 384 healthy individuals and 162 individuals that had pathological impairments in their sensory systems were tested. When we analyzed data the from the cohort of healthy individuals we noted a considerable variation in the test results (**Figures 7**, **8**, **9**, **10**) and asked how much of this was age dependent variation. It is a common notion that the performance of both hearing and vision systems decrease with age. As expected, this was the case for the three hearing traits investigated in our study (**Figure 8**). In addition, we found that most of the other sensory traits examined in our study were also age dependent. Vibration detection threshold and tactile acuity showed strong age dependence with older people having higher thresholds (**Figure 7**). This is in accordance with previous studies where younger cohorts showed a better performance in many touch related tasks (Wickremaratchi and Llewelyn 2006; Shaffer and Harrison 2007). Deteriorating performances were also shown specifically for grating orientation tasks (Stevens and Patterson 1995; Stevens and Cruz 1996; Sathian, Zangaladze et al. 1997) and have been shown in numerous studies examining vibration sensitivity (Verrillo 1980; Merchut and Toleikis 1990; Meh and Denislic 1995; Goble, Collins et al. 1996; Lin, Hsieh et al. 2005). An age related decrease in the number of cutaneous receptor

structures, as has been shown for Pacinian and Meissner's corpuscles (Cauna and Mannan 1958; Bolton, Winkelmann et al. 1966; Bruce 1980), has been proposed as the reason for increasing thresholds in age.

The cardiovascular system is affected by age in many ways and the effect of age on the baroreflex has been studied extensively (Monahan 2007). As expected, we saw a strong age related decrease in the baroreflex slopes determined by the sequence technique and by cross spectral analysis (**Figure 9A-C**). The baroreflex sequence frequency is not normally considered in a clinical evaluation of baroreflex function and to our knowledge has not been studied for age dependence so far. However it is the only trait, of all investigated, that showed no significant age dependence (**Figure 9D**).

All temperature sensitivity traits, including heat and cold pain thresholds showed significant age dependence in this study, with higher temperature change detection thresholds and higher temperature pain thresholds (**Figure 10**) in older individuals. Previous studies that addressed the effect of age on temperature change thresholds as well as thermal pain thresholds are contradictory. Temperature change thresholds showed an age dependent threshold increase in some studies (Doeland, Nauta et al. 1989; Stevens and Choo 1998; Lautenbacher, Kunz et al. 2005; Lin, Hsieh et al. 2005; Huang, Wang et al. 2010) whereas it did not in others (Hilz, Stemper et al. 1999; Harju 2002; Litscher, Wang et al. 2004; Lin, Hsieh et al. 2005). For heat pain the situation is even more ambiguous because there are reports of decreasing thresholds in age (Norbury, MacGregor et al. 2007; Huang, Wang et al. 2010) as well as unchanged thresholds (Lautenbacher and Strian 1991; Litscher, Wang et al. 2004; Lautenbacher, Kunz et al. 2005) and increasing thresholds (Sherman and Robillard 1964). For cold pain, increases in thresholds with age have been reported (Meh and Denislic 1994; Huang, Wang et al. 2010). It is hard to see a reason for all these differences in the results since there are differences in the procedures between the studies, but their overall design is quite similar. However, the detected age dependence of temperature sensitivity was robust in our study.

5.2 Gender differences in sensory traits

The analysis of gender effects on sensory traits gave a mixed picture. Around half of the investigated traits, such as tactile acuity, showed significant gender dependence, whereas the others, such as vibration detection threshold, did not (**Figure 11**). However, compared to the effect of age, gender effects were much smaller. We could not detect a significant gender difference in vibration detection thresholds (**Figure 11A**), which has been reported before (Meh and Denislic 1995; Lin, Hsieh et al. 2005), whereas other studies reported equivocal results (Hilz, Stemper et al. 1999; Lindsell and Griffin 2003) or reported a higher sensitivity of female test persons. A small, but significant, difference could be seen in the tactile acuity test (**Figure 11B**): women displayed better tactile acuity performance than men, in agreement with previous studies (Goldreich and Kanics 2003; Peters, Hackeman et al. 2009). Interestingly, the gender effect could be completely eliminated in the study of Peters et al. (Peters, Hackeman et al. 2009) when results were controlled for finger size, suggesting a constant number of receptors independent of finger size. A higher density of receptors, primarily Merkel's discs in this case, would have a particularly strong effect on the tactile acuity threshold, where spatial resolution, as well as the intensity of skin indentation, play a role.

Hearing acuity did not show a significant gender effect in our study (**Figure 11C-E**). A gender effect is well described in the literature (Corso 1963; McFadden 1993; Pearson, Morrell et al. 1995; Murphy and Gates 1997; Borchgrevink, Tambs et al. 2005), but these differences are frequency dependent. Women have a higher acuity at high frequencies above 2 kHz and men are slightly more sensitive at the lower frequencies, especially when measured in older cohorts. Considering that in our study frequency thresholds between 0.5 kHz and 4 kHz were averaged, the lack of gender effect was not unexpected. In our analysis of both reproducibility and strength of the otoacoustic emissions, women had higher mean values, which has been observed in all studies that addressed this matter that we are aware of (Ferguson, Smith et al. 2000; Engdahl 2002; Johansson and Arlinger 2003; Shahnaz 2008; McFadden, Martin et al. 2009; Pavlovcinova, Jakubikova et al. 2010). Gender differences of cochlear function exist from birth and could be attributed to anatomical differences of the ear, e.g. a longer male cochlea (Sato, Sando et al. 1991).

Hearing sensitivity is modulated by the influence of sex hormones as is evident by the fact that hearing acuity in females is changes over the menstrual cycle (Swanson and Dengerink 1988).

No significant gender effect was detectable in the three measures of the baroreflex slope (**Figure F-H**). Previous reports are conflicting, some show no difference between genders (Linden and Diehl 1996; Tank, Baevski et al. 2000) or report inconsistent results, (Virtanen, Jula et al. 2004), whereas others report a lower baroreflex response in women, at least in the respiratory (high frequency) domain (Huikuri, Pikkujamsa et al. 1996; Kardos, Watterich et al. 2001; Dietrich, Riese et al. 2006; Barantke, Krauss et al. 2008). Most reports are concerned with the strength of the baroreflex reaction. We also analyzed the baroreflex sequence frequency, that is, how often a baroreflex reaction is detectable in a resting person over a period of 5 min. Here we saw a significant gender effect with a higher frequency for women (**Figure 11I**).

While we found women to be significantly more sensitive in the temperature change detection tests (**Figure 11J**, **K**), no difference could be detected for the thermal pain thresholds (**Figure 11L, M**). As for the age dependence of temperature sensitivity traits, the reports about gender differences are contradictory. Temperature change detection thresholds were reported unchanged in males compared to females in some studies (Harju 2002; Litscher, Wang et al. 2004) others reported higher sensitivity of women in both warmth and cold detection thresholds (Doeland, Nauta et al. 1989; Huang, Wang et al. 2010) and two studies found the warmth detection threshold lower in women and the cold detection threshold unchanged (Lautenbacher and Strian 1991; Lin, Hsieh et al. 2005). Again for the heat pain threshold all possible results appear in the literature, with studies where no gender effects were observed (Lautenbacher and Strian 1991; Fillingim and Maixner 1996), lower thresholds in women were observed (Meh and Denislic 1994) or lower thresholds in men (Kenshalo 1986). Previous studies examining cold pain thresholds showed either lower thresholds (less cooling required to reach threshold) in women (Meh and Denislic 1994; Litscher, Wang et al. 2004) or no gender difference (Liou, Lui et al. 1999).

5.3 The heritability of sensory traits

In this study we report for the first time a genetic influence on the variability of touch sensitivity in humans. We could estimate a heritability value of 0.52 (0.33 - 0.67; 95 % CI) for the vibration detection threshold and of 0.27 (0.05 – 0.46; 95 % CI) for tactile acuity (**Figure 13**). That tells us that around half of the variation observed in the vibration detection test and around a quarter of the variation observed in the tactile acuity test is determined by the stochastic genetic composition of each individual. The heritability value for the vibration detection threshold is rather high and very robust as can be seen by the narrow 95 % confidence interval, whereas the heritability value of the tactile acuity is less robust. In both cases, as well as for all other traits for which heritability values could be estimated, the common environment component was not included in the preferred model (**AE** – model). This was expected, since the relatively low number of 100 twin pairs does not provide enough power for estimating a more complex model (Neale 2004).
A lower heritability of tactile acuity in a grating orientation test is plausible, if we consider the following arguments. In contrast to a simple detection threshold, further processing in the central nervous system is required to determine the grating orientation in space. Even though neurons that respond specifically to certain orientations of a tactile stimulus can be found in the primary somatosensory cortex (Bensmaia, Denchev et al. 2008), other brain areas involved in multisensory processing are also active during grating orientation tasks (Van Boven, Ingeholm et al. 2005; Zhang, Mariola et al. 2005; Kitada, Kito et al. 2006). Furthermore, activation (Sathian, Zangaladze et al. 1997), and even requirement of the visual cortex for performing grating orientation tasks (Zangaladze, Epstein et al. 1999), has been reported. These processing steps provide neural plasticity that allows unique environmental factors and training to alter the individual's performance, which in turn would lead to a decrease in heritability. In contrast, this amount of processing is not required for vibration detection. The activity of single cutaneous sensory fibers is sufficient to be perceived as the respective sensory modality, e.g. vibration (Vallbo 1981; Ochoa and Torebjork 1983; Vallbo, Olsson et al. 1984; Torebjork, Vallbo et al. 1987). In a number of studies the psychophysical threshold were compared to the neural thresholds as determined by microneurography (Konietzny

and Hensel 1977; Johansson and Vallbo 1979; Jarvilehto, Hamalainen et al. 1981). For some fibers the psychophysical thresholds were identical to the neural thresholds, whereas in other fibers, especially outside the most sensitive regions of the hand and the high frequency detecting (Pacinian) fibers, the psychophysical thresholds were higher. Here, temporal summation is required to reach psychophysical threshold levels (Verrillo 1965; Green 1976; Gescheider and Joelson 1983). However, thresholds to generate somatosensory evoked potentials, as determined by EEG recordings, were shown to be in the same range as psychophysical thresholds (Soininen and Jarvilehto 1983). Compared to the tactile acuity test, the vibration detection threshold test may therefore be considered more indicative of the actual sensitivity of cutaneous mechanoreceptors.

Another factor that has to be considered, apart from actual differences in heritability, is the design of the tests. In the tactile acuity test the distribution of thresholds is covered by only 5 groove width steps on the tactile acuity cube, whilst there are fifteen amplitude steps in the vibration detection threshold test, meaning a higher resolution for the latter test. The different resolution of the two tests, together with the fact that the tactile acuity test was performed manually, may lead to a lower reproducibility of the tactile acuity test. This lower reproducibility was confirmed in preparative experiments (data not shown), where the same cohort was tested twice. Since measurement error is included in the unique environment component of the heritability model, heritability estimates will be lower if the accuracy of the test is lower. Fully automated grating orientation task test devices are in use (Goldreich, Wong et al. 2009), but test duration and mobility were considered when tests for this study were chosen.

Our study showed that there is a large heritable component to touch sensitivity, and that we can approach touch sensitivity on a genetic level with standardized, commercially available equipment for quantitative sensory testing. This makes these tests eligible for use in genome-wide approaches to identify quantitative trait loci (QTLs) and ultimately genes that are involved in touch sensitivity. This could be association studies or linkage studies, the latter combined with an extension of the twin study. If a trait is highly heritable it does not automatically mean that there are single gene variants that account for a large proportion of the variance of the trait (Maher 2008; Yang, Benyamin et al. 2010). However, the standardization and the short duration of the tests (~ 15 min per test)

could make testing of larger, already genotyped cohorts, possible. We hypothesized the existence of a common genetic basis of touch sensitivity traits and other mechanosensory traits, which can be demonstrated with the classical twin study design. However, in this relatively small sample of 100 twin pairs, the detection of a genetic correlation of different mechanosensitivity traits could not be expected because of lack of power. (Neale 2004). If a genetic correlation could be detected in an extended twin study, incorporation of the respective trait in a genome-wide association / linkage study, in addition to tactile acuity and the vibration detection threshold, would further enhance the power of a multivariate analysis.

Hearing has been subject to twin studies before, but in these studies the focus was exclusively on hearing acuity in elderly populations and age related hearing loss (Christensen, Frederiksen et al. 2001; Viljanen, Era et al. 2007; Wingfield, Panizzon et al. 2007), a factor specifically excluded from our analysis. To our knowledge this is the first report about the heritability in a general population excluding age as a factor. The high heritability value of 0.8 (**Figure 14**) might be surprising, considering that even short term exposure to high noise levels can inflict irreparable damage to the ear, but even for age related hearing loss, heritability values well above 0.5 have been reported (Christensen, Frederiksen et al. 2001; Viljanen, Era et al. 2007; Wingfield, Panizzon et al. 2007). Heritability estimates for both measures of the otoacoustic emissions were also very high, which is in accordance with a previous study on the heritability of otoacoustic emissions (McFadden, Loehlin et al. 1996).

We detected robust heritability in all the measures of baroreflex except for the low baroreflex slope in the low frequency domain (**Figure 15**). Since all three values determined for the baroreflex slope are describing the same parameter, the difference is likely due to measurement error. Heritability values for all three measures of baroreflex slope have been determined before (Tank, Jordan et al. 2001) and here a robust genetic contribution was shown for baroreflex slope in the low frequency domain. This difference could be due to the higher number of twin pairs tested in the previous study of Tank et al.. The baroreflex sequence frequency was not analysed for heritability by Tank et al.. It showed the highest heritability value of the four baroreflex measures.

Our finding of a robust heritability of temperature sensitivity (**Figure 16A, B**) is the first report of a genetic component in the sensitivity to innocuous temperatures. A genetic component has been reported before for the heat pain threshold (Norbury, MacGregor et al. 2007), whereas we could not detect a heritable component for the heat or cold pain thresholds (**Figure 16C**, **D**). The heat pain threshold is the only one of the investigated traits that shows a higher cross-twin correlation for DZ twin pairs compared to MD twin pairs. As for the age and gender dependence it is difficult to determine why the studies on heat pain thresholds produce contradictory results, especially considering that in this case study size and testing equipment were identical. However, one factor may be that only women were tested in the study of Norbury et al. (Norbury, MacGregor et al. 2007).

5.4 Phenotypical correlations between sensory traits

We analyzed the cross-correlations between the sensory traits within all healthy individuals that were tested from the entire cohort (**Table 5**). As expected, traits of the same modality showed high correlations. In the analysis of correlations between the different sensory systems, only one significant correlation was detected between a temperature sensitivity trait and one of the mechanosensory traits, namely between warmth detection threshold and hearing acuity. In contrast, there were four significant correlations between traits of different mechanosensory systems (**Figure 17**). Tactile acuity correlated with hearing acuity and the EOAE reproducibility, hearing acuity also correlated with the low frequency baroreflex slope and the baroreflex sequence frequency with the EAOE strength. Therefore, at least on a phenotypic level, the different mechanosensory traits appear to be related. The correlations are all rather small, with correlation coefficients between 0.16 and 0.22, therefore in this case the data was analyzed again after a correction was made for gender differences, where such differences were present. After this correction, only the correlation between the hearing acuity and tactile acuity, as well as the low frequency baroreflex slope, were present (**Table 6**). Of course, this does not tell us if these correlations originate from the existence of common mechanisms in sensory transduction or because of common central pathways. We also do not know if these similarities can be attributed to the influence of

the same genes. A genetic correlation could be determined in an extended twin study, as discussed above, and since phenotypic correlation is prerequisite for this, hearing acuity is good candidate for being included in such a study.

5.5 Touch sensitivity and congenital hearing impairment

In our cohort of hearing impaired individuals, we observed a significantly lower touch sensitivity, as measured in tactile acuity and the vibration detection threshold tests (**Figure 18**). From this result we cannot tell whether the attenuation in touch sensitivity results from common peripheral transduction mechanisms or if it is because of an effect on the central nervous system, caused by sensory deprivation. Central interplay between touch processing and auditory processing has been reported before, as shown by the activation of the auditory cortex by tactile stimuli (Caetano and Jousmaki 2006; Schurmann, Caetano et al. 2006). The fact that central auditory pathways are used in processing of tactile information does not tell us that processing of tactile information would be impaired by the lack of auditory input. It has been shown that the auditory cortex is even more active in response to tactile stimuli in deaf test persons compared to normal hearing individuals (Auer, Bernstein et al. 2007). The individuals with the ten highest thresholds in the tactile acuity test are not the same individuals with the ten highest thresholds in the vibration detection threshold test. If a central deficiency caused by sensory deprivation would cause reduced touch sensitivity, we would expect similar effects in all individuals and not impairments in specific parameters of touch sensitivity, as observed in our study.

We can only speculate about the composition of our cohort in respect to the question of whether hereditary hearing loss is the cause of the hearing impairment and what genes are actually involved. However, in most cases congenital deafness is caused by a genetic defect (Smith, Bale et al. 2005). As a next step it is now planned to identify mutations in a cohort of congenital deaf / hearing impaired individuals by means of next generation sequencing based targeted resequencing and relate the identified mutations to touch sensitivity measures. This will be conducted using a cohort that has already tested negative for a mutation in the gene *Connexin26*. Mutations in *Connexin26* are responsible

for most cases of hereditary hearing loss. This can be up to 50 % of the cases, but varies greatly between populations (Apps, Rankin et al. 2007). Since *Connexin26* is not expressed in hair cells, it is not a candidate for being involved directly in the mechanotransduction process.

5.6 Touch sensitivity and the Usher syndrome

There are nine genes known that cause the Usher syndrome when they are mutated. We could show reduced touch sensitivity in cohorts of patients that carry pathogenic mutations in either the gene *Myosin7a* (*Myo7a*) or *Ush2a*.

The mean vibration detection threshold was significantly higher in the cohort of people carrying a mutation in the gene *Myo7a* (**Figure 20A**). The role of Myo7a, which causes Usher syndrome type 1, when mutated, in humans, has been studied in the hair cells in wild type mice and mice that carry mutations in the *Myo7a* gene, the shaker-1 mice. As with other Usher proteins, MYO7A is located in the stereocilia of the hair cells (Hasson, Heintzelman et al. 1995; Senften, Schwander et al. 2006). The MYO7A protein binds directly to other Usher proteins, e.g. Harmonin, Protocadherin15 and Cadherin23 (Senften, Schwander et al. 2006) and has been shown to be required for the proper localization of other Usher proteins (Boeda, El-Amraoui et al. 2002; Michalski, Michel et al. 2007). The stereocilia bundles of shaker-1 mice are disorganized (Holme and Steel 2002) and longer compared to wild type mice (Prosser, Rzadzinska et al. 2008) indicating a role of Myosin7a in controlling actin dynamics. However, transduction currents can still be recorded in shaker-1 mice (Kros, Marcotti et al. 2002), but the stereocila bundle has to be deflected more than it would happen under physiological conditions to elicit a current. Also the adaptation kinetics of the current is changed. The shaker-1 mice have also been tested by our group for alterations of their touch sensitivity (Milenkovic, Frenzel unpublished). Transduction currents measured in cultured DRG neurons showed prolonged adaptation kinetics. Extracellular recordings from cutaneous sensory nerve fibers revealed that the low threshold mechanoreceptor fibers desensitized almost completely following repeated stimulation. Shaker-1 mice were also tested on a behavioral level and it was shown that the ability of the mice to detect gratings embossed

in the floor was severely reduced. Therefore, there is evidence that MYO7A is involved in the mechanotransduction in the hair cells as well as in the cutaneous sensory neurons in mice and in humans.

The second group of Usher type 1 patients carried mutations in the *Cadherin23* gene (*Cdh23*). This cohort did not show changed cutaneous mechanosensitivity (**Figure 20C, D**). Cadherin23 has been shown to be part of the tip link, the structure that connects the tip of the hair cells stereocilia with the adjacent, longer stereocilia (Siemens, Kazmierczak et al. 2002; Kazmierczak, Sakaguchi et al. 2007). The tip link is thought to exert mechanical tension on the mechanotransduction complex, located on the tips of the stereocilia (Beurg, Fettiplace et al. 2009), when the stereocilia bundle is deflected. A tether-like structure similar to the tip links has been detected on cutaneous sensory neurons and it has been shown that this structure is required for mechanosensitivity (Hu, Chiang et al. 2010). However, CDH23 is not thought to be component of this structure since the cutaneous tether is susceptible to degradation by the enzymes furin and blisterase. These enzymes are specific to certain amino acid sequences that are not present in the extracellular parts of CDH23 (Hu, Chiang et al. 2010).

In the cohort of people carrying mutations in the gene *Ush2a* (also called *Usherin*), causing Usher syndrome type 2, we found a significantly reduced tactile acuity (**Figure 21B, D)**. USH2A is transmembrane protein with a large extracellular domain (van Wijk, Pennings et al. 2004). The USH2A protein is localized to the base of the stereocilia and is thought to be part of the ankle links, that connect adjecent stereocilia at their bases (Adato, Lefevre et al. 2005; Michalski, Michel et al. 2007). USH2A could be detected only in the developing hair cell of the cochlea, but also at later stages in vestibular hair cells. USH2A has been shown to bind to other Usher proteins (Adato, Lefevre et al. 2005; Reiners, van Wijk et al. 2005) as well as to collagen IV (Bhattacharya, Kalluri et al. 2004) and could be a link between the inner network of Usher proteins and the extracellular matrix. Stereocilial bundles are disorganized in mice mutated in the *Ush2a* gene to varying extents, increasingly so towards the distal end of the cochlea. A similar role in organizing and / or maintaining structures required for mechanotransduction is also conceivable in cutaneous sensory neurons. At this stage, it is hard to see why only tactile acuity, a trait that is mainly reliant on slowly adapting sensory fibers, is affected. It

would further be of great interest to test USH2A deficient mice for possible deficits in cutaneous mechanotransduction and touch related behavior.

For 24 test persons Usher syndrome type 2 has been clinically diagnosed, but no information about molecular genetics was available. This group did not show a significant difference in touch sensitivity (**Figure 21E, F**). The fact that not all groups of Usher patients show reduced touch sensitivity and that different aspects of touch sensitivity are concerned in the affected groups argues against a central effect caused by sensory deprivation. In this case a more uniform effect on touch sensitivity might be expected.

5.7 Baroreflex function and the Usher syndrome

Baroreflex function was tested in individuals carrying mutations in *Ush2a* and in Usher syndrome type 2 patients without genotypic information and no differences were found (**Figure 22**). This does not rule out that one or more of the other eight known Usher genes could have an effect. Considering the phenotypic correlation between baroreflex slope and hearing acuity (**Figure 17**) baroreflex testing in Usher patients should be continued, especially Usher syndrome type 1 patients that have not been tested at all so far.

5.8 Temperature sensitivity and the Usher syndrome

Temperature sensitivity was tested in individuals carrying mutations in *Ush2a* and in Usher syndrome type 2 patients with unknown mutations (**Figure 23**). Here we found decreased thresholds for warmth, as well as cold detection thresholds in the group carrying mutations in *Ush2a*. We can only speculate about the underlying mechanism. USH2A protein has been shown to be located not only in the stereocilia of the hair cells, but also at the synapse (van Wijk, van der Zwaag et al. 2006). If this would be also true for sensory neurons, one could think of an enhanced synaptic transmission in the spinal cord caused by mutations in USH2A. If we would assume that enhanced temperature sensitivity could be caused by compensation for the reduction of sensory input from the

auditory and from the vision system, we would expect both groups tested to be affected in the same way but this was not the case. However, the fact that the temperature detection thresholds are not lower in the Usher syndrome type 2 patient cohorts, compared to control cohorts, excludes that a general impairment of the cutaneous sensory system is responsible for the observed reduction of touch sensitivity in patients with mutations in the *Ush2a* gene (**Figure 21**).

5.9 Touch sensitivity and blindness

We tested a cohort of Braille reading blind individuals for their touch sensitivity. Tactile acuity was clearly enhanced in blind people compared to sighted controls, whereas the vibration detection threshold was unchanged (**Figure 24**). Despite the common belief that blind people are superior in tactile tasks, there are surprisingly few reports in the literature about this subject and the results are conflicting. Blind people have been found to perform better in some, but not all tactile tests. Better performance of the blind has been reported for dot pattern determination, grating detection and texture discrimination tasks (Grant, Thiagarajah et al. 2000; Goldreich and Kanics 2006; Legge, Madison et al. 2008; Alary, Duquette et al. 2009) and no difference between blind and sighted cohorts has been reported for grating width determination and frequency determination tasks (Grant, Thiagarajah et al. 2000; Alary, Duquette et al. 2009). Grating orientation tasks have been employed in multiple studies where some report a better performance of the blind (Van Boven, Hamilton et al. 2000; Goldreich and Kanics 2003) and others report no increased performance in the blind (Grant, Thiagarajah et al. 2000; Alary, Duquette et al. 2009).

The detected increase in tactile acuity in our study was highly significant although we could not find a difference between the main Braille reading index finger and the contralateral index finger. This is in accordance with the previous observation that tactile learning is systemic and transfers between fingers, but is specific to certain task (Sathian and Zangaladze 1997). It should be noted that, in contrast to our findings, van Boven et al. found a difference between the main Braille reading finger and the contralateral finger (Van Boven, Hamilton et al. 2000).

The results from the study on touch sensitivity in blind people also complement the results from the twin study, where we found that the variation of tactile acuity is less determined by genetic influences compared to the vibration detection threshold. By studying tactile acuity in blind people we have shown that tactile acuity is more susceptible to enhancement by learning, since both tactile acuity and vibration sensitivity are required for active tactile processes as Braille reading, but only the former was enhanced in the cohort of blind people.

5.10 Screening of candidate genes for involvement in peripheral mechanotransduction

In order to identify genes that are involved in mechanotransduction we matched the expression profiles of candidate genes in sensory neurons to the development of mechanosensitivity in the mouse.

We screened a number of genes that have been discussed in the literature to play a role in mechanotransduction, or other sensory processes, for their co-regulation with the onset of mechanosensitivity in developing DRG neurons. Most of these genes did not fulfill the chosen criteria of upregulation between embryonic stages E 11.5 and E 14.5 and sustained expression afterwards (**Table 9**). Some TRP ion channels, TRPC1, TRPV2, TRPV4 and TRPM7 are activated by stretch (Liedtke, Choe et al. 2000; Strotmann, Harteneck et al. 2000; Muraki, Iwata et al. 2003; Maroto, Raso et al. 2005; Numata, Shimizu et al. 2007). If one of these stretch activated channels were the mechanotransducer in the cutanoeus sensory neurons, expression of the channels alone would be sufficient for generating transduction currents expression and would be especially tightly coupled to transduction current emergence in development. The expression of these channels before emergence of mechanosensitivity, as shown by us, makes their involvement seem unlikely.

Another group of ion channels that has been suggested to be involved in mechanotransduction is the degenerin / epithelial sodium channel (DEG/ENaC) superfamily. Involvement in touch sensitivity in mice has been demonstrated for two of these ion channels, ASIC2 and ASIC3 (Price, Lewin et al. 2000; Price, McIlwrath et al.

2001). The expression profile of these ASIC channels fitted our criteria and although a role as transduction channels has been challenged (Drew, Rohrer et al. 2004) the respective knock-out mouse models have been tested again in our group by Dr. Stefan Lechner and no alteration of transduction currents was observed (Lechner, Frenzel et al. 2009).

Two other genes that matched the desired expression profile of a mechanotransduction gene code for the ion channels TRPM8 and TRPV1. These ion channels convey thermal and chemical sensitivity in the cutaneous sensory system (Bautista, Siemens et al. 2007; Caterina 2007; Dhaka, Murray et al. 2007) and are thus not promising candidates as mechanotransducers.

No role in cutaneous sensation has been proposed so far for the other genes fulfilling the screening criteria. These are the genes TRP channel genes *Trpc4*, *Trpm2* and *Trpm3*, the "deafness –gene" *Myo1a* and *Slp1*, a gene coding for a protein homologous to *Slp3*. Slp3 is the only protein shown to be required specifically for mechanotransduction in mice (Wetzel, Hu et al. 2007). A knock-out mouse model for SLP1 generated by Dr. Alexey Kozlenkov is currently being investigated by our group. There are knockout models for TRPM2 and TRPC4, but no obvious tactile deficits were reported. *Trpc4* was strongly upregulated in the extended screen employing cultured sensory neurons (**Figure 28**). Considering the availability of the TRPC4 knockout, an investigation of mechanotransduction currents in these mice seems appropriate.

Of the nine Usher genes, all were found to be expressed in the DRGs, some at comparably low levels, yet none fulfilled our expression criteria. But if we consider that USH2A is only detectable in the developing cochlear hair cells (see above) and might have its primary role in establishing structures required for mechanotransduction rather than being a functional part of them, the expression profile appears more fitting. *Ush2a* expression fits our criterion of upregulation between E 11.5 and E 14.5 but is downregulated again in the DRGs of adult mice.

5.11 Genome-wide screen for genes involved in peripheral mechanotransduction

In the genome-wide expression profiling screen using Affymetrix expression arrays, we looked for genes that were not expressed at E 11.5, but at E14.5 and still higher at the adult stage and that contained at least one transmembrane domain. Fifty-three genes emerged as candidates after confirmation by quantitative PCR (**Table 10**). Among those were genes coding for transporters, ABC-transporters, cell-adhesion proteins and structural proteins but also 15 genes of unknown function. One additional observation about the development of mechanosensitivity can be taken into account to further reduce the list of candidates before functional testing in knock-down or knock-out experiments begin: the first cells requiring mechanosensitivity around embryonic stage E 13.5 are the cells with the largest cell bodies. Genes that are only expressed in large cells at this stage would be most promising candidates for being involved in mechanosensitivity. Expression patterns can be tested by in-situ hybridizations of DRGs either as a whole or in sections.

It has to be noted that in this screen we assume a gene to be functional when a gene is expressed in terms of mRNA levels. Presence of mRNA does not automatically mean that there also is functional protein. Translation can be regulated as can the function of a synthesized protein. The stage chosen as non-mechanosensitive is actually one and a half to two days before actual emergence of mechanosensitivity and it is unlikely that transcription should start this long before the functional emergence. It has been shown for other proteins with sensory properties in cutaneous sensory neurons that emergence of mRNA and functional detection are tightly coupled (Hjerling-Leffler, Alqatari et al. 2007).

5.12 Conclusions

There is a genetic component in the variability of two different aspects of touch sensitivity, tactile acuity and vibration detection threshold. The genetic influence of touch sensitivity is assessable by standardized equipment used in clinical quantitative sensory testing. We found a phenotypic correlation between mechanosensitivity traits of different modalities.

Touch sensitivity is impaired in some forms of congenital hearing impairment, but not in others. Reduced sensitivity could be specifically related to mutations in the genes *Myo7a* (reduced vibration sensitivity) and *Ush2a* (reduced tactile acuity). In a cohort of blind people, tactile acuity was found to be enhanced, whereas vibration sensitivity was unchanged.

6 References

Adato, A., G. Lefevre, et al. (2005). "Usherin, the defective protein in Usher syndrome type IIA, is likely to be a component of interstereocilia ankle links in the inner ear sensory cells." Hum Mol Genet **14**(24): 3921-3932.

Adriaensen, H., J. Gybels, et al. (1983). "Response properties of thin myelinated (A-delta) fibers in human skin nerves." J Neurophysiol **49**(1): 111-122.

Ahmed, Z. M., R. Goodyear, et al. (2006). "The tip-link antigen, a protein associated with the transduction complex of sensory hair cells, is protocadherin-15." J Neurosci **26**(26): 7022-7034.

Ahmed, Z. M., S. Riazuddin, et al. (2003). "PCDH15 is expressed in the neurosensory epithelium of the eye and ear and mutant alleles are responsible for both USH1F and DFNB23." Hum Mol Genet **12**(24): 3215-3223.

Ahmed, Z. M., T. N. Smith, et al. (2002). "Nonsyndromic recessive deafness DFNB18 and Usher syndrome type IC are allelic mutations of USHIC." Hum Genet **110**(6): 527-531.

Alary, F., M. Duquette, et al. (2009). "Tactile acuity in the blind: a closer look reveals superiority over the sighted in some but not all cutaneous tasks." Neuropsychologia **47**(10): 2037-2043.

Apps, S. A., W. A. Rankin, et al. (2007). "Connexin 26 mutations in autosomal recessive deafness disorders: a review." Int J Audiol **46**(2): 75-81.

Auer, E. T., Jr., L. E. Bernstein, et al. (2007). "Vibrotactile activation of the auditory cortices in deaf versus hearing adults." Neuroreport **18**(7): 645-648.

Barantke, M., T. Krauss, et al. (2008). "Effects of gender and aging on differential autonomic responses to orthostatic maneuvers." J Cardiovasc Electrophysiol **19**(12): 1296-1303.

Bautista, D. M., S. E. Jordt, et al. (2006). "TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents." Cell **124**(6): 1269-1282.

Bautista, D. M., J. Siemens, et al. (2007). "The menthol receptor TRPM8 is the principal detector of environmental cold." Nature **448**(7150): 204-208.

Beck, P. W., H. O. Handwerker, et al. (1974). "Nervous outflow from the cat's foot during noxious radiant heat stimulation." Brain Res **67**(3): 373-386.

Bensmaia, S. J., P. V. Denchev, et al. (2008). "The representation of stimulus orientation in the early stages of somatosensory processing." J Neurosci **28**(3): 776-786.

Bertinieri, G., M. di Rienzo, et al. (1985). "A new approach to analysis of the arterial baroreflex." J Hypertens Suppl **3**(3): S79-81.

Bessou, P. and E. R. Perl (1969). "Response of cutaneous sensory units with unmyelinated fibers to noxious stimuli." J Neurophysiol **32**(6): 1025-1043.

Beurg, M., R. Fettiplace, et al. (2009). "Localization of inner hair cell mechanotransducer channels using high-speed calcium imaging." Nat Neurosci **12**(5): 553-558.

Bhattacharya, G. and D. Cosgrove (2005). "Evidence for functional importance of usherin/fibronectin interactions in retinal basement membranes." Biochemistry **44**(34): 11518-11524.

Bhattacharya, G., R. Kalluri, et al. (2004). "A domain-specific usherin/collagen IV interaction may be required for stable integration into the basement membrane superstructure." J Cell Sci **117**(Pt 2): 233-242.

Bhattacharya, G., C. Miller, et al. (2002). "Localization and expression of usherin: a novel basement membrane protein defective in people with Usher's syndrome type IIa." Hear Res **163**(1-2): 1-11.

Boeda, B., A. El-Amraoui, et al. (2002). "Myosin VIIa, harmonin and cadherin 23, three Usher I gene products that cooperate to shape the sensory hair cell bundle." EMBO J **21**(24): 6689-6699.

Bolton, C. F., R. K. Winkelmann, et al. (1966). "A quantitative study of Meissner's corpuscles in man." Neurology **16**(1): 1-9.

Bolz, H., S. S. Bolz, et al. (2004). "Impaired calmodulin binding of myosin-7A causes autosomal dominant hearing loss (DFNA11)." Hum Mutat **24**(3): 274-275.

Borchgrevink, H. M., K. Tambs, et al. (2005). "The Nord-Trondelag Norway Audiometric Survey 1996-98: unscreened thresholds and prevalence of hearing impairment for adults > 20 years." Noise Health **7**(28): 1-15.

Bork, J. M., L. M. Peters, et al. (2001). "Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene CDH23." Am J Hum Genet **68**(1): 26-37.

Bounoutas, A. and M. Chalfie (2007). "Touch sensitivity in Caenorhabditis elegans." Pflugers Arch **454**(5): 691-702.

Brown, A. M., W. R. Saum, et al. (1976). "A comparison of aortic baroreceptor discharge in normotensive and spontaneously hypertensive rats." Circ Res **39**(4): 488-496.

Brownell, W. E., C. R. Bader, et al. (1985). "Evoked mechanical responses of isolated cochlear outer hair cells." Science **227**(4683): 194-196.

Bruce, M. F. (1980). "The relation of tactile thresholds to histology in the fingers of elderly people." J Neurol Neurosurg Psychiatry **43**(8): 730-734.

Burgess, P. R. and E. R. Perl (1967). "Myelinated afferent fibres responding specifically to noxious stimulation of the skin." J Physiol **190**(3): 541-562.

Caetano, G. and V. Jousmaki (2006). "Evidence of vibrotactile input to human auditory cortex." Neuroimage **29**(1): 15-28.

Caterina, M. J. (2007). "Transient receptor potential ion channels as participants in thermosensation and thermoregulation." Am J Physiol Regul Integr Comp Physiol **292**(1): R64-76.

Cauna, N. and G. Mannan (1958). "The structure of human digital pacinian corpuscles (corpus cula lamellosa) and its functional significance." J Anat **92**(1): 1-20.

Christensen, K., H. Frederiksen, et al. (2001). "Genetic and environmental influences on self-reported reduced hearing in the old and oldest old." J Am Geriatr Soc **49**(11): 1512-1517.

Cioffi, D. L., K. Lowe, et al. (2009). "TRPing on the lung endothelium: calcium channels that regulate barrier function." Antioxid Redox Signal **11**(4): 765-776.

Cohen, M. A. and J. A. Taylor (2002). "Short-term cardiovascular oscillations in man: measuring and modelling the physiologies." J Physiol **542**(Pt 3): 669-683.

Coleridge, H. M., J. C. Coleridge, et al. (1987). "Characteristics of C fibre baroreceptors in the carotid sinus of dogs." J Physiol **394**: 291-313.

Corey, D. P. and A. J. Hudspeth (1983). "Kinetics of the receptor current in bullfrog saccular hair cells." J Neurosci **3**(5): 962-976.

Corso, J. F. (1963). "Age and sex differences in pure-tone thresholds. Survey of hearing levels from 18 to 65 years." Arch Otolaryngol **77**: 385-405.

Crawford, A. C., M. G. Evans, et al. (1989). "Activation and adaptation of transducer currents in turtle hair cells." J Physiol **419**: 405-434.

Crawford, A. C. and R. Fettiplace (1985). "The mechanical properties of ciliary bundles of turtle cochlear hair cells." J Physiol **364**: 359-379.

Dallos, P. (1992). "The active cochlea." J Neurosci **12**(12): 4575-4585.

Darian-Smith, I., K. O. Johnson, et al. (1973). ""Cold" fiber population innervating palmar and digital skin of the monkey: responses to cooling pulses." J Neurophysiol **36**(2): 325-346.

Darian-Smith, I., K. O. Johnson, et al. (1979). "Warm fibers innervating palmar and digital skin of the monkey: responses to thermal stimuli." J Neurophysiol **42**(5): 1297-1315.

Davis, J. B., J. Gray, et al. (2000). "Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia." Nature **405**(6783): 183-187.

deBoer, R. W., J. M. Karemaker, et al. (1987). "Hemodynamic fluctuations and baroreflex sensitivity in humans: a beat-to-beat model." Am J Physiol **253**(3 Pt 2): H680-689.

Dhaka, A., A. N. Murray, et al. (2007). "TRPM8 is required for cold sensation in mice." Neuron **54**(3): 371-378.

Dhaka, A., V. Viswanath, et al. (2006). "Trp ion channels and temperature sensation." Annu Rev Neurosci **29**: 135-161.

Dietrich, A., H. Riese, et al. (2006). "Spontaneous baroreflex sensitivity in (pre)adolescents." J Hypertens **24**(2): 345-352.

Doeland, H. J., J. J. Nauta, et al. (1989). "The relationship of cold and warmth cutaneous sensation to age and gender." Muscle Nerve **12**(9): 712-715.

Donaudy, F., A. Ferrara, et al. (2003). "Multiple mutations of MYO1A, a cochlear-expressed gene, in sensorineural hearing loss." Am J Hum Genet **72**(6): 1571-1577.

Drew, L. J., D. K. Rohrer, et al. (2004). "Acid-sensing ion channels ASIC2 and ASIC3 do not contribute to mechanically activated currents in mammalian sensory neurones." J Physiol **556**(Pt 3): 691-710.

Drummond, H. A., M. J. Welsh, et al. (2001). "ENaC subunits are molecular components of the arterial baroreceptor complex." Ann N Y Acad Sci **940**: 42-47.

Dubner, R., R. Sumino, et al. (1975). "A peripheral "cold" fiber population responsive to innocuous and noxious thermal stimuli applied to monkey's face." J Neurophysiol **38**(6): 1373-1389.

Duclaux, R. and D. R. Kenshalo, Sr. (1980). "Response characteristics of cutaneous warm receptors in the monkey." J Neurophysiol **43**(1): 1-15.

Dyck, P. J., I. R. Zimmerman, et al. (1978). "Introduction of automated systems to evaluate touch-pressure, vibration, and thermal cutaneous sensation in man." Ann Neurol **4**(6): 502-510.

Eberl, D. F., R. W. Hardy, et al. (2000). "Genetically similar transduction mechanisms for touch and hearing in Drosophila." J Neurosci **20**(16): 5981-5988.

Ebermann, I., H. P. Scholl, et al. (2007). "A novel gene for Usher syndrome type 2: mutations in the long isoform of whirlin are associated with retinitis pigmentosa and sensorineural hearing loss." Hum Genet **121**(2): 203-211.

Edin, B. B. (1992). "Quantitative analysis of static strain sensitivity in human mechanoreceptors from hairy skin." J Neurophysiol **67**(5): 1105-1113.

Engdahl, B. (2002). "Otoacoustic emissions in the general adult population of Nord-Trondelag, Norway: I. Distributions by age, gender, and ear side." Int J Audiol **41**(1): 64-77.

Ferguson, M. A., P. A. Smith, et al. (2000). "Transient-evoked otoacoustic emissions in a representative population sample aged 18 to 25 years." Audiology **39**(3): 125-134.

Fettiplace, R. (2006). "Active hair bundle movements in auditory hair cells." J Physiol **576**(Pt 1): 29-36.

Fidone, S. J. and A. Sato (1969). "A study of chemoreceptor and baroreceptor A and C-fibres in the cat carotid nerve." J Physiol **205**(3): 527-548.

Fillingim, R. B. and W. Maixner (1996). "The influence of resting blood pressure and gender on pain responses." Psychosom Med **58**(4): 326-332.

Fonfria, E., I. C. Marshall, et al. (2004). "TRPM2 channel opening in response to oxidative stress is dependent on activation of poly(ADP-ribose) polymerase." Br J Pharmacol **143**(1): 186-192.

Georgopoulos, A. P. (1976). "Functional properties of primary afferent units probably related to pain mechanisms in primate glabrous skin." J Neurophysiol **39**(1): 71-83.

Gescheider, G. A. and J. M. Joelson (1983). "Vibrotactile temporal summation for threshold and suprathreshold levels of stimulation." Percept Psychophys **33**(2): 156-162.

Goble, A. K., A. A. Collins, et al. (1996). "Vibrotactile threshold in young and old observers: the effects of spatial summation and the presence of a rigid surround." J Acoust Soc Am **99**(4 Pt 1): 2256-2269.

Goldreich, D. and I. M. Kanics (2003). "Tactile acuity is enhanced in blindness." J Neurosci **23**(8): 3439-3445.

Goldreich, D. and I. M. Kanics (2006). "Performance of blind and sighted humans on a tactile grating detection task." Percept Psychophys **68**(8): 1363-1371.

Goldreich, D., M. Wong, et al. (2009). "A Tactile Automated Passive-Finger Stimulator (TAPS)." J Vis Exp(28).

Grant, A. C., M. C. Thiagarajah, et al. (2000). "Tactile perception in blind Braille readers: a psychophysical study of acuity and hyperacuity using gratings and dot patterns." Percept Psychophys **62**(2): 301-312.

Green, B. G. (1976). "Vibrotactile temporal summation: effect of frequency." Sens Processes **1**(2): 138-149.

Gruener, G. and P. J. Dyck (1994). "Quantitative sensory testing: methodology, applications, and future directions." J Clin Neurophysiol **11**(6): 568-583.

Harju, E. L. (2002). "Cold and warmth perception mapped for age, gender, and body area." Somatosens Mot Res **19**(1): 61-75.

Hasson, T., M. B. Heintzelman, et al. (1995). "Expression in cochlea and retina of myosin VIIa, the gene product defective in Usher syndrome type 1B." Proc Natl Acad Sci U S A **92**(21): 9815-9819.

Heiner, I., J. Eisfeld, et al. (2003). "Role and regulation of TRP channels in neutrophil granulocytes." Cell Calcium **33**(5-6): 533-540.

Hilz, M. J., B. Stemper, et al. (1999). "Quantitative thermal perception testing in adults." J Clin Neurophysiol **16**(5): 462-471.

Hjerling-Leffler, J., M. Alqatari, et al. (2007). "Emergence of functional sensory subtypes as defined by transient receptor potential channel expression." J Neurosci **27**(10): 2435-2443.

Holme, R. H. and K. P. Steel (2002). "Stereocilia defects in waltzer (Cdh23), shaker1 (Myo7a) and double waltzer/shaker1 mutant mice." Hear Res **169**(1-2): 13-23.

Hu, J., L. Y. Chiang, et al. (2010). "Evidence for a protein tether involved in somatic touch." EMBO J **29**(4): 855-867.

Hu, J. and G. R. Lewin (2006). "Mechanosensitive currents in the neurites of cultured mouse sensory neurones." J Physiol **577**(Pt 3): 815-828.

Huang, H. W., W. C. Wang, et al. (2010). "Influence of age on thermal thresholds, thermal pain thresholds, and reaction time." J Clin Neurosci **17**(6): 722-726.

Hudspeth, A. J. and D. P. Corey (1977). "Sensitivity, polarity, and conductance change in the response of vertebrate hair cells to controlled mechanical stimuli." Proc Natl Acad Sci U S A **74**(6): 2407-2411.

Huikuri, H. V., S. M. Pikkujamsa, et al. (1996). "Sex-related differences in autonomic modulation of heart rate in middle-aged subjects." Circulation **94**(2): 122-125.

Iggo, A. (1960). "Cutaneous mechanoreceptors with afferent C fibres." J Physiol **152**: 337-353.

Jarvilehto, T., H. Hamalainen, et al. (1981). "Peripheral neural basis of tactile sensations in man: II. Characteristics of human mechanoreceptors in the hairy skin and correlations of their activity with tactile sensations." Brain Res **219**(1): 13-27.

Johansson, M. S. and S. D. Arlinger (2003). "Otoacoustic emissions and tympanometry in a general adult population in Sweden." Int J Audiol **42**(8): 448-464.

Johansson, R. S. and A. B. Vallbo (1979). "Detection of tactile stimuli. Thresholds of afferent units related to psychophysical thresholds in the human hand." J Physiol **297**(0): 405-422.

Johnson, K. O., T. Yoshioka, et al. (2000). "Tactile functions of mechanoreceptive afferents innervating the hand." J Clin Neurophysiol **17**(6): 539-558.

Kardos, A., G. Watterich, et al. (2001). "Determinants of spontaneous baroreflex sensitivity in a healthy working population." Hypertension **37**(3): 911-916.

Kaupp, U. B. (2010). "Olfactory signalling in vertebrates and insects: differences and commonalities." Nat Rev Neurosci **11**(3): 188-200.

Kazmierczak, P., H. Sakaguchi, et al. (2007). "Cadherin 23 and protocadherin 15 interact to form tip-link filaments in sensory hair cells." Nature **449**(7158): 87-91.

Kemp, D. T. (1978). "Stimulated acoustic emissions from within the human auditory system." J Acoust Soc Am **64**(5): 1386-1391.

Kemp, D. T., P. Bray, et al. (1986). "Acoustic emission cochleography--practical aspects." Scand Audiol Suppl **25**: 71-95.

Kenshalo, D. R. and R. Duclaux (1977). "Response characteristics of cutaneous cold receptors in the monkey." J Neurophysiol **40**(2): 319-332.

Kenshalo, D. R., Sr. (1986). "Somesthetic sensitivity in young and elderly humans." J Gerontol **41**(6): 732-742.

Kim, J., Y. D. Chung, et al. (2003). "A TRPV family ion channel required for hearing in Drosophila." Nature **424**(6944): 81-84.

Kitada, R., T. Kito, et al. (2006). "Multisensory activation of the intraparietal area when classifying grating orientation: a functional magnetic resonance imaging study." J Neurosci **26**(28): 7491-7501.

Knibestol, M. and A. B. Vallbo (1980). "Intensity of sensation related to activity of slowly adapting mechanoreceptive units in the human hand." J Physiol **300**: 251-267.

Konietzny, F. and H. Hensel (1977). "Response of rapidly and slowly adapting mechanoreceptors and vibratory sensitivity in human hairy skin." Pflugers Arch **368**(1-2): 39-44.

Koushanpour (1991). "Baroreceptor Discharge Behavior and Resetting." In: Persson P.B.: Baroreceptor reflexes: 9-44.

Kremer, H., E. van Wijk, et al. (2006). "Usher syndrome: molecular links of pathogenesis, proteins and pathways." Hum Mol Genet **15 Spec No 2**: R262-270.

Kros, C. J., W. Marcotti, et al. (2002). "Reduced climbing and increased slipping adaptation in cochlear hair cells of mice with Myo7a mutations." Nat Neurosci **5**(1): 41-47.

Kwan, K. Y., J. M. Glazer, et al. (2009). "TRPA1 modulates mechanotransduction in cutaneous sensory neurons." J Neurosci **29**(15): 4808-4819.

Lautenbacher, S., M. Kunz, et al. (2005). "Age effects on pain thresholds, temporal summation and spatial summation of heat and pressure pain." Pain **115**(3): 410-418.

Lautenbacher, S. and F. Strian (1991). "Sex differences in pain and thermal sensitivity: the role of body size." Percept Psychophys **50**(2): 179-183.

Lautenbacher, S. and F. Strian (1991). "Similarities in age differences in heat pain perception and thermal sensitivity." Funct Neurol **6**(2): 129-135.

Lechner, S. G., H. Frenzel, et al. (2009). "Developmental waves of mechanosensitivity acquisition in sensory neuron subtypes during embryonic development." EMBO J **28**(10): 1479-1491.

Legge, G. E., C. Madison, et al. (2008). "Retention of high tactile acuity throughout the life span in blindness." Percept Psychophys **70**(8): 1471-1488.

Lewin, G. R. and R. Moshourab (2004). "Mechanosensation and pain." J Neurobiol **61**(1): 30-44.

Liedtke, W., Y. Choe, et al. (2000). "Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor." Cell **103**(3): 525-535.

Lin, S. Y. and D. P. Corey (2005). "TRP channels in mechanosensation." Curr Opin Neurobiol **15**(3): 350-357.

Lin, Y. H., S. C. Hsieh, et al. (2005). "Influence of aging on thermal and vibratory thresholds of quantitative sensory testing." J Peripher Nerv Syst **10**(3): 269-281.

Linden, D. and R. R. Diehl (1996). "Estimation of baroreflex sensitivity using transfer function analysis: normal values and theoretical considerations." Clin Auton Res **6**(3): 157-161.

Lindsell, C. J. and M. J. Griffin (2003). "Normative vibrotactile thresholds measured at five European test centres." Int Arch Occup Environ Health **76**(7): 517-528.

Liou, J. T., P. W. Lui, et al. (1999). "Normative data of quantitative thermal and vibratory thresholds in normal subjects in Taiwan: gender and age effect." Zhonghua Yi Xue Za Zhi (Taipei) **62**(7): 431-437.

Litscher, G., L. Wang, et al. (2004). "[Quantification of gender specific thermal sensory and pain threshold before and after laser needle stimulation]." Biomed Tech (Berl) **49**(5): 106-110.

Liu, X., O. V. Bulgakov, et al. (2007). "Usherin is required for maintenance of retinal photoreceptors and normal development of cochlear hair cells." Proc Natl Acad Sci U S A **104**(11): 4413-4418.

Loewenstein, W. R. and R. Rathkamp (1958). "The sites for mechano-electric conversion in a Pacinian corpuscle." J Gen Physiol **41**(6): 1245-1265.

Lu, Y., X. Ma, et al. (2009). "The ion channel ASIC2 is required for baroreceptor and autonomic control of the circulation." Neuron **64**(6): 885-897.

Lumpkin, E. A. and M. J. Caterina (2007). "Mechanisms of sensory transduction in the skin." Nature **445**(7130): 858-865.

Macefield, V. G. (2005). "Physiological characteristics of low-threshold mechanoreceptors in joints, muscle and skin in human subjects." Clin Exp Pharmacol Physiol **32**(1-2): 135-144.

Macefield, V. G., C. Hager-Ross, et al. (1996). "Control of grip force during restraint of an object held between finger and thumb: responses of cutaneous afferents from the digits." Exp Brain Res **108**(1): 155-171.

Maher, B. (2008). "Personal genomes: The case of the missing heritability." Nature **456**(7218): 18-21.

Mairhofer, M., M. Steiner, et al. (2009). "Stomatin-like protein-1 interacts with stomatin and is targeted to late endosomes." J Biol Chem **284**(42): 29218-29229.

Maroto, R., A. Raso, et al. (2005). "TRPC1 forms the stretch-activated cation channel in vertebrate cells." Nat Cell Biol **7**(2): 179-185.

Mburu, P., M. Mustapha, et al. (2003). "Defects in whirlin, a PDZ domain molecule involved in stereocilia elongation, cause deafness in the whirler mouse and families with DFNB31." Nat Genet **34**(4): 421-428.

McCarter, G. C., D. B. Reichling, et al. (1999). "Mechanical transduction by rat dorsal root ganglion neurons in vitro." Neurosci Lett **273**(3): 179-182.

McFadden, D. (1993). "A speculation about the parallel ear asymmetries and sex differences in hearing sensitivity and otoacoustic emissions." Hear Res **68**(2): 143-151.

McFadden, D. and J. C. Loehlin (1995). "On the heritability of spontaneous otoacoustic emissions: A twins study." Hear Res **85**(1-2): 181-198.

McFadden, D., J. C. Loehlin, et al. (1996). "Additional findings on heritability and prenatal masculinization of cochlear mechanisms: click-evoked otoacoustic emissions." Hear Res **97**(1-2): 102-119.

McFadden, D., G. K. Martin, et al. (2009). "Sex differences in distortion-product and transient-evoked otoacoustic emissions compared." J Acoust Soc Am **125**(1): 239-246.

McKemy, D. D., W. M. Neuhausser, et al. (2002). "Identification of a cold receptor reveals a general role for TRP channels in thermosensation." Nature **416**(6876): 52-58.

Meh, D. and M. Denislic (1994). "Quantitative assessment of thermal and pain sensitivity." J Neurol Sci **127**(2): 164-169.

Meh, D. and M. Denislic (1995). "Influence of age, temperature, sex, height and diazepam on vibration perception." J Neurol Sci **134**(1-2): 136-142.

Merchut, M. P. and S. C. Toleikis (1990). "Aging and quantitative sensory thresholds." Electromyogr Clin Neurophysiol **30**(5): 293-297.

Michalski, N., V. Michel, et al. (2007). "Molecular characterization of the ankle-link complex in cochlear hair cells and its role in the hair bundle functioning." J Neurosci **27**(24): 6478-6488.

Mills, L. R. and J. Diamond (1995). "Merkel cells are not the mechanosensory transducers in the touch dome of the rat." J Neurocytol **24**(2): 117-134.

Monahan, K. D. (2007). "Effect of aging on baroreflex function in humans." Am J Physiol Regul Integr Comp Physiol **293**(1): R3-R12.

Moqrich, A., S. W. Hwang, et al. (2005). "Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin." Science **307**(5714): 1468-1472.

Mountcastle, V. B., R. H. LaMotte, et al. (1972). "Detection thresholds for stimuli in humans and monkeys: comparison with threshold events in mechanoreceptive afferent nerve fibers innervating the monkey hand." J Neurophysiol **35**(1): 122-136.

Muller, M. (1991). "Frequency representation in the rat cochlea." Hear Res **51**(2): 247-254.

Muraki, K., Y. Iwata, et al. (2003). "TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes." Circ Res **93**(9): 829-838.

Murphy, M. P. and G. A. Gates (1997). "Hearing Loss: Does Gender Play a Role?" Medscape Womens Health **2**(10): 2.

Neale, M. C. (2004). Methodology for genetic studies of twins and families.

Norbury, T. A., A. J. MacGregor, et al. (2007). "Heritability of responses to painful stimuli in women: a classical twin study." Brain **130**(Pt 11): 3041-3049.

Numata, T., T. Shimizu, et al. (2007). "Direct mechano-stress sensitivity of TRPM7 channel." Cell Physiol Biochem **19**(1-4): 1-8.

Ochoa, J. and E. Torebjork (1983). "Sensations evoked by intraneural microstimulation of single mechanoreceptor units innervating the human hand." J Physiol **342**: 633-654.

Pavlovcinova, G., J. Jakubikova, et al. (2010). "A normative study of otoacoustic emissions, ear asymmetry, and gender effect in healthy schoolchildren in Slovakia." Int J Pediatr Otorhinolaryngol **74**(2): 173-177.

Pearson, J. D., C. H. Morrell, et al. (1995). "Gender differences in a longitudinal study of age-associated hearing loss." J Acoust Soc Am **97**(2): 1196-1205.

Peters, R. M., E. Hackeman, et al. (2009). "Diminutive digits discern delicate details: fingertip size and the sex difference in tactile spatial acuity." J Neurosci **29**(50): 15756-15761.

Phillips, J. R. and K. O. Johnson (1981). "Tactile spatial resolution. II. Neural representation of Bars, edges, and gratings in monkey primary afferents." J Neurophysiol **46**(6): 1192-1203.

Pickles, J. O., S. D. Comis, et al. (1984). "Cross-links between stereocilia in the guinea pig organ of Corti, and their possible relation to sensory transduction." Hear Res **15**(2): 103-112.

Price, M. P., G. R. Lewin, et al. (2000). "The mammalian sodium channel BNC1 is required for normal touch sensation." Nature **407**(6807): 1007-1011.

Price, M. P., S. L. McIlwrath, et al. (2001). "The DRASIC cation channel contributes to the detection of cutaneous touch and acid stimuli in mice." Neuron **32**(6): 1071-1083.

Price, M. P., R. J. Thompson, et al. (2004). "Stomatin modulates gating of acid-sensing ion channels." J Biol Chem **279**(51): 53886-53891.

Prosser, H. M., A. K. Rzadzinska, et al. (2008). "Mosaic complementation demonstrates a regulatory role for myosin VIIa in actin dynamics of stereocilia." Mol Cell Biol **28**(5): 1702-1712.

Purves, D., Ed. (2001). Neuroscience 2nd ed. Sunderland (MA, USA), Sinauer Associates.

Rees, P. M. (1967). "Observations on the fine structure and distribution of presumptive baroreceptor nerves at the carotid sinus." J Comp Neurol **131**(4): 517-548.

Reiners, J., E. van Wijk, et al. (2005). "Scaffold protein harmonin (USH1C) provides molecular links between Usher syndrome type 1 and type 2." Hum Mol Genet **14**(24): 3933-3943.

Rivolta, C., E. A. Sweklo, et al. (2000). "Missense mutation in the USH2A gene: association with recessive retinitis pigmentosa without hearing loss." Am J Hum Genet **66**(6): 1975-1978.

Roper, S. D. (2007). "Signal transduction and information processing in mammalian taste buds." Pflugers Arch **454**(5): 759-776.

Sano, Y., K. Inamura, et al. (2001). "Immunocyte Ca2+ influx system mediated by LTRPC2." Science **293**(5533): 1327-1330.

Sathian, K. and A. Zangaladze (1997). "Tactile learning is task specific but transfers between fingers." Percept Psychophys **59**(1): 119-128.

Sathian, K., A. Zangaladze, et al. (1997). "Tactile spatial acuity and roughness discrimination: impairments due to aging and Parkinson's disease." Neurology **49**(1): 168-177.

Sathian, K., A. Zangaladze, et al. (1997). "Feeling with the mind's eye." Neuroreport **8**(18): 3877-3881.

Sato, H., I. Sando, et al. (1991). "Sexual dimorphism and development of the human cochlea. Computer 3-D measurement." Acta Otolaryngol **111**(6): 1037-1040.

Sato, M. (1961). "Response of Pacinian corpuscles to sinusoidal vibration." J Physiol **159**: 391-409.

Schurmann, M., G. Caetano, et al. (2006). "Touch activates human auditory cortex." Neuroimage **30**(4): 1325-1331.

Seller (1991). "Central Baroreceptor Reflex Pathways." In: Persson P.B.: Baroreceptor reflexes: 45-75.

Sellick, P. M., R. Patuzzi, et al. (1982). "Measurement of basilar membrane motion in the guinea pig using the Mossbauer technique." J Acoust Soc Am **72**(1): 131-141.

Senften, M., M. Schwander, et al. (2006). "Physical and functional interaction between protocadherin 15 and myosin VIIa in mechanosensory hair cells." J Neurosci **26**(7): 2060-2071.

Shaffer, S. W. and A. L. Harrison (2007). "Aging of the somatosensory system: a translational perspective." Phys Ther **87**(2): 193-207.

Shahnaz, N. (2008). "Transient evoked otoacoustic emissions (TEOAEs) in Caucasian and Chinese young adults." Int J Audiol **47**(2): 76-83.

Sherman, E. D. and E. Robillard (1964). "Sensitivity to Pain in Relationship to Age." J Am Geriatr Soc **12**: 1037-1044.

Shukla, G., M. Bhatia, et al. (2005). "Quantitative thermal sensory testing -- value of testing for both cold and warm sensation detection in evaluation of small fiber neuropathy." Clin Neurol Neurosurg **107**(6): 486-490.

Siemens, J., P. Kazmierczak, et al. (2002). "The Usher syndrome proteins cadherin 23 and harmonin form a complex by means of PDZ-domain interactions." Proc Natl Acad Sci U S A **99**(23): 14946-14951.

Simone, D. A. and K. C. Kajander (1997). "Responses of cutaneous A-fiber nociceptors to noxious cold." J Neurophysiol **77**(4): 2049-2060.

Smith, R. J., J. F. Bale, Jr., et al. (2005). "Sensorineural hearing loss in children." Lancet **365**(9462): 879-890.

Soc.Pacing-Electrophysiology (1996). "Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology." Circulation **93**(5): 1043-1065.

Soininen, K. and T. Jarvilehto (1983). "Somatosensory evoked potentials associated with tactile stimulation at detection threshold in man." Electroencephalogr Clin Neurophysiol **56**(5): 494-500.

Stauffer, E. A., J. D. Scarborough, et al. (2005). "Fast adaptation in vestibular hair cells requires myosin-1c activity." Neuron **47**(4): 541-553.

Stevens, J. C. and K. K. Choo (1998). "Temperature sensitivity of the body surface over the life span." Somatosens Mot Res **15**(1): 13-28.

Stevens, J. C. and L. A. Cruz (1996). "Spatial acuity of touch: ubiquitous decline with aging revealed by repeated threshold testing." Somatosens Mot Res **13**(1): 1-10.

Stevens, J. C. and M. Q. Patterson (1995). "Dimensions of spatial acuity in the touch sense: changes over the life span." Somatosens Mot Res **12**(1): 29-47.

Strotmann, R., C. Harteneck, et al. (2000). "OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity." Nat Cell Biol **2**(10): 695-702.

Stuart, M., A. B. Turman, et al. (2003). "Effects of aging on vibration detection thresholds at various body regions." BMC Geriatr **3**: 1.

Sung, C. H. and J. Z. Chuang (2010). "The cell biology of vision." J Cell Biol **190**(6): 953-963.

Swanson, S. J. and H. A. Dengerink (1988). "Changes in pure-tone thresholds and temporary threshold shifts as a function of menstrual cycle and oral contraceptives." J Speech Hear Res **31**(4): 569-574.

Tank, J., R. M. Baevski, et al. (2000). "Reference values of indices of spontaneous baroreceptor reflex sensitivity." Am J Hypertens **13**(3): 268-275.

Tank, J., J. Jordan, et al. (2001). "Genetic influences on baroreflex function in normal twins." Hypertension **37**(3): 907-910.

Togashi, K., Y. Hara, et al. (2006). "TRPM2 activation by cyclic ADP-ribose at body temperature is involved in insulin secretion." EMBO J **25**(9): 1804-1815.

Tominaga, M., M. J. Caterina, et al. (1998). "The cloned capsaicin receptor integrates multiple pain-producing stimuli." Neuron **21**(3): 531-543.

Torebjork, H. E. and R. G. Hallin (1974). "Identification of afferent C units in intact human skin nerves." Brain Res **67**(3): 387-403.

Torebjork, H. E., A. B. Vallbo, et al. (1987). "Intraneural microstimulation in man. Its relation to specificity of tactile sensations." Brain **110 (Pt 6)**: 1509-1529.

Tyska, M. J., A. T. Mackey, et al. (2005). "Myosin-1a is critical for normal brush border structure and composition." Mol Biol Cell **16**(5): 2443-2457.

Vallbo, A. B. (1981). "Sensations evoked from the glabrous skin of the human hand by electrical stimulation of unitary mechanosensitive afferents." Brain Res **215**(1-2): 359-363.

Vallbo, A. B., H. Olausson, et al. (1995). "Receptive field characteristics of tactile units with myelinated afferents in hairy skin of human subjects." J Physiol **483 (Pt 3)**: 783-795.

Vallbo, A. B., K. A. Olsson, et al. (1984). "Microstimulation of single tactile afferents from the human hand. Sensory attributes related to unit type and properties of receptive fields." Brain **107 (Pt 3)**: 727-749.

Van Boven, R. W., R. H. Hamilton, et al. (2000). "Tactile spatial resolution in blind braille readers." Neurology **54**(12): 2230-2236.

Van Boven, R. W., J. E. Ingeholm, et al. (2005). "Tactile form and location processing in the human brain." Proc Natl Acad Sci U S A **102**(35): 12601-12605.

Van Boven, R. W. and K. O. Johnson (1994). "The limit of tactile spatial resolution in humans: grating orientation discrimination at the lip, tongue, and finger." Neurology **44**(12): 2361-2366.

van Wijk, E., R. J. Pennings, et al. (2004). "Identification of 51 novel exons of the Usher syndrome type 2A (USH2A) gene that encode multiple conserved functional domains and that are mutated in patients with Usher syndrome type II." Am J Hum Genet **74**(4): 738-744.

van Wijk, E., B. van der Zwaag, et al. (2006). "The DFNB31 gene product whirlin connects to the Usher protein network in the cochlea and retina by direct association with USH2A and VLGR1." Hum Mol Genet **15**(5): 751-765.

Verrillo, R. T. (1965). "Temporal Summation in Vibrotactile Sensitivity." J Acoust Soc Am **37**: 843-846.

Verrillo, R. T. (1980). "Age related changes in the sensitivity to vibration." J Gerontol **35**(2): 185-193.

Viljanen, A., P. Era, et al. (2007). "Genetic and environmental influences on hearing in older women." J Gerontol A Biol Sci Med Sci **62**(4): 447-452.

Virtanen, R., A. Jula, et al. (2004). "Increased pulse pressure is associated with reduced baroreflex sensitivity." J Hum Hypertens **18**(4): 247-252.

Wagner, T. F., S. Loch, et al. (2008). "Transient receptor potential M3 channels are ionotropic steroid receptors in pancreatic beta cells." Nat Cell Biol **10**(12): 1421-1430.

Wan, C. Y., A. G. Wood, et al. (2010). "Congenital blindness leads to enhanced vibrotactile perception." Neuropsychologia **48**(2): 631-635.

Werner, G. and V. B. Mountcastle (1965). "Neural Activity in Mechanoreceptive Cutaneous Afferents: Stimulus-Response Relations, Weber Functions, and Information Transmission." J Neurophysiol **28**: 359-397.

Wetzel, C., J. Hu, et al. (2007). "A stomatin-domain protein essential for touch sensation in the mouse." Nature **445**(7124): 206-209.

Wickremaratchi, M. M. and J. G. Llewelyn (2006). "Effects of ageing on touch." Postgrad Med J **82**(967): 301-304.

Willems, E., I. Mateizel, et al. (2006). "Selection of reference genes in mouse embryos and in differentiating human and mouse ES cells." Int J Dev Biol **50**(7): 627-635.

Wingfield, A., M. Panizzon, et al. (2007). "A twin-study of genetic contributions to hearing acuity in late middle age." J Gerontol A Biol Sci Med Sci **62**(11): 1294-1299.

Wood, J. N. (2004). "Recent advances in understanding molecular mechanisms of primary afferent activation." Gut **53 Suppl 2**: ii9-12.

Woodbury, C. J., M. Zwick, et al. (2004). "Nociceptors lacking TRPV1 and TRPV2 have normal heat responses." J Neurosci **24**(28): 6410-6415.

Wu, D., W. Huang, et al. (2008). "TRPC4 in rat dorsal root ganglion neurons is increased after nerve injury and is necessary for neurite outgrowth." J Biol Chem **283**(1): 416-426.

Yang, J., B. Benyamin, et al. (2010). "Common SNPs explain a large proportion of the heritability for human height." Nat Genet **42**(7): 565-569.

Yao, T. and P. Thoren (1983). "Characteristics of brachiocephalic and carotid sinus baroreceptors with non-medullated afferents in rabbit." Acta Physiol Scand **117**(1): 1-8.

Zangaladze, A., C. M. Epstein, et al. (1999). "Involvement of visual cortex in tactile discrimination of orientation." Nature **401**(6753): 587-590.

Zhang, M., E. Mariola, et al. (2005). "Tactile discrimination of grating orientation: fMRI activation patterns." Hum Brain Mapp **25**(4): 370-377.

Zheng, J., W. Shen, et al. (2000). "Prestin is the motor protein of cochlear outer hair cells." Nature **405**(6783): 149-155.

Zwislocki, J. J. and E. M. Relkin (2001). "On a psychophysical transformed-rule up and down method converging on a 75% level of correct responses." Proc Natl Acad Sci U S A **98**(8): 4811-4814.