Published in final edited form as:
Arch Oral Biol. 2008 November ; 53(11): 1058–1070. doi:10.1016/j.archoralbio.2008.05.006.

Age and Gender Related Differences in Human Parotid Gland Gene Expression

Alaka Srivastava[a,c], **Jianghua Wang**[b,c], **Hui Zhou**[b], **James E. Melvin**[a,*], and **David T. Wong**[b]

a*Center for Oral Biology and the Department of Pharmacology and Physiology, University of Rochester Medical Center, Rochester, NY 14642*

b*Department of Oral Biology and Oral Medicine, School of Dentistry and Dental Research Institute, University of California, Los Angeles*

Abstract

Objective—The present study evaluated differences in gene expression associated with age and gender in the human parotid gland.

Design—Parotid gland tissue was analyzed using the Affymetrix® GeneChip® HGU133plus2.0 array.

Results—Differential gene expression, defined as a statistically significant difference with a 1.5 fold or greater change, was detected in 787 gene probe sets; 467 (~59%) showed higher expression in females. Several genes associated with saliva secretion were differentially expressed in male and female parotid glands including vesicle-associated membrane protein 3 VAMP3, synaptosomal-associated protein SNAP23, RAS oncogene family member RAB1A and the syntaxin binding protein STXBP1. Evaluation of gene expression in the youngest and the oldest female subjects revealed that the expression of 228 probe sets were altered during aging; 155 genes were up-regulated in the aged female parotid gland. However, of the genes that were altered during aging, 22 of the 30 probes (73%) classified as being associated with immune responses were down-regulated in the aged parotid gland. A panel of differentially expressed, age- and gender-related genes was selected for validation by quantitative, real-time RT-PCR. Comparable differences in gene expression were detected by both Affymetrix array and quantitative, real-time RT-PCR methods.

Conclusions—Our data suggest that salivary gland function may be adversely affected in the aged population due, at least in part, to the altered regulation of several categories of genes. Moreover, the gender specific differences in gene expression identified in the present study correlate with the previously observed sexual dimorphism in salivary gland function.

Keywords

Human parotid; Salivary gland; Gene expression

*Corresponding author Dr. James E. Melvin: Center of Oral Biology, medical Center Box 611, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY 14642. Email: james_melvin@urmc.rochester.edu.
[c]Contributed equally

INTRODUCTION

Previous studies suggest that there may be age and gender related differences in salivary gland function (1–5). However, the limited and often conflicting information available from healthy populations makes it difficult to confirm these differences (6–9). Saliva is produced and secreted into the oral cavity by the exocrine salivary glands. Humans possess three major pairs of salivary glands (parotid, submandibular and sublingual) and several types of minor salivary glands scattered throughout the oral cavity. The majority of saliva (>80%) is generated by the two largest of these glands, the parotid and submandibular glands (10,11). Recent studies show that saliva contains well over one thousand different unique proteins, the functions for the majority of which are yet to be determined (12).

Salivation is a highly regulated process which occurs at a relatively slow rate between meals, with almost no secretion during sleep (13). The importance of saliva to oral health is most evident in subjects suffering from severe salivary gland hypofunction commonly associated with the autoimmune disease Sjögren's syndrome, radiation therapy of head and neck cancers, and numerous types of medications. In these cases, there is a dramatic increase in both oral and systemic disease (14). Thus, without adequate saliva output, oral and pharyngeal health declines along with quality of life.

Persistent dry mouth is also a common symptom in aged individuals, although the mechanisms involved are not well understood. Dry mouth in the elderly is frequently associated with the increased use of medications and the functional disturbances associated with these medications. Both age and gender associated differences in the structure and function of salivary glands have been identified (1,4). Examples of such differences include a decrease in gland size and weight (15), decreased saliva flow rate (1–3), and an increased concentration of immunoglobulin A (IgA) (3). In humans, decreases in protein synthesis (16) and salivary flow rate (1,2,4,5) have been reported. Given these age related differences in salivary gland structure and function, it is expected that significant changes in gene expression must occur. However, other studies have failed to demonstrate a relationship between age and decreased function (6–9). Thus, the limited and often conflicting information available from healthy populations makes it difficult to confirm these differences. The purpose of the present study was to evaluate and compare differences in gene expression associated with age and gender in the human parotid gland.

MATERIALS and METHODS

Human Parotid Gland Tissue

Human parotid glands were obtained from 32 otherwise healthy male (n=13) and female (n=19) subjects (19–85 years of age) scheduled to have parotid surgery because their gland contained a benign tumor that required removal of all or a large portion of the gland. All samples were pathologically confirmed to be benign salivary gland tumors (pleomorphic adenoma, mixed tumor or Warthin's tumor). Subjects were excluded who indicated that they experienced dry mouth or took medications known to adversely affect salivary gland function. Much of the normal tissue surrounding the tumor is not used for diagnostic evaluation of the sample. This discarded tissue was collected immediately after surgical excision and transported in ice-cold physiological saline to the laboratory where the tissue was frozen in liquid N_2. Tissue was obtained and used as approved by the University of Rochester Institutional Review Board, or in the case of 3 samples, obtained through the Cooperative Human Tissue Network (CHTN). The functional properties of the parotid tissue obtained by these criteria appear normal in all respects (17,18).

RNA Isolation and Array Analysis

Total RNA from parotid gland tissues was treated with RNase free DNase (Qiagen) and isolated by affinity chromatography according to the manufacturer's protocol (RNeasy kit (Qiagen, Valencia, CA). RNA (3 μg) was subjected to 1 round of linear amplification with the RiboAmp™ RNA Amplification kit (ARCTURUS, Mountain View CA) and biotinylated using GeneChip® Expression 3'-Amplification reagents for IVT labeling (Affymetrix). Before hybridization, 13 μg of labeled RNA was fragmented using 5X fragmentation Buffer (Affymetrix). RNA quality was monitored before and after amplification, as well after fragmentation (2100 bioanalyzer, Agilent Technologies). Hybridization to the Human Genome U133 Plus 2.0 Array as well as imagine scanning (Affymetrix, Santa Clara, CA, USA) was performed by the Microarray Core Facility at the University of California, Los Angeles, according to standard protocols provided by Affymetrix.

Array Data and Statistic Analysis

The fluorescence intensities of the arrays were measured by Array Suite 5.0 software (Affymetrix). The data were imported into DNA-Chip Analyzer software (Affymetrix) for normalization and model-based analysis (19). A detection *p*-value was obtained for each probe set, and any probe sets with $p < 0.04$ were assigned as a "present" call, indicating that the matching gene transcript was reliably detected (Statistical algorithms description document. Affymetrix, 2002). The raw data were then exported to Microsoft Excel software for data sorting and mining.

The GeneSifter® array data analysis system (VizX Labs LLC, Seattle, WA) was used to identify age and gender related differences in gene expression of the human parotid gland. The Affymetrix non-normalized data (CEL files) were transferred into GeneSifter. Expression measurements were derived using RMA (robust multiple average) and filtering criteria of a 1.5 or greater fold change (20). Statistical significance was determined by Student's t-test, and data were corrected for multiple testing using the method of Benjamini and Hochberg (21). Only the probe sets which passed the quality filtering with *p* values <0.05 were included in the analysis.

Pathway Analysis

Genes with significantly different expression were overlaid using GeneSifter software onto Ontological pathways (http://www.geneontology.org/) (22) and KEGG pathways (http://www.genome.jp/kegg/) (23). The ontological and KEGG pathway analyses provided detailed data on individual genes in the context of that gene's role in described biological processes, molecular functions, and cellular components. Pathways were considered significantly altered from the control gene expression profiles if the *z*-score for that pathway was less than −2 or greater than 2. *z*-Scores were calculated in GeneSifter using the following formula:

$$\text{Z-score} = \frac{(r - n)\frac{R}{N}}{\sqrt{N(\frac{R}{N})(1 - \frac{R}{N})(1 - \frac{n-1}{N-1})}}$$

where R = total number of genes meeting selection criteria, N = total number of genes measured, r = number of genes meeting selection criteria with the specified GO term, and n = total number of genes measured with the specific GO term (24). The data from the individual arrays (n = 13) are accessible for download through the National Center for Biotechnology Information's Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) through series accession number (GSE8764).

RT-PCR and Q-PCR

Quantitative PCR (Q-PCR) was used to validate microarray results. First-strand complementary DNA (cDNA) was synthesized from 1 μg of total RNA in a 20 μl reaction using the iScript™ cDNA synthesis kit (Bio-Rad, Hercules, CA). A primer set for each gene was generated using *GeneFisher* software (http://bibiserv.techfak.uni-bielefeld.de/genefisher/). The primers were synthesized commercially (Integrated DNA Technologies, Coralville, IA). Q-PCR was performed in triplicate using a 96-well iCycler IQ™ Real Time PCR Detection System (Bio-Rad, Hercules, CA) in 25 μl total volume containing 12.5 μl of 2XSYBR Green Master Mix , 0.5 to 1.0 μl of cDNA and 0.5 μl and 2 ng/μl of gene specific primers (Table 1). Q-PCR amplification was carried out by 40 cycles of 30 sec at 95°C and 30 sec at 60°C. Dissociation curves were monitored (60°C for 10 sec to 95°C in 0.5°C/10 sec increments) to ensure the absence of secondary PCR product. The predicted sizes of the PCR products were verified by agarose gel electrophoresis. In most cases, the PCR products were also sequence verified. The endogenous control, L32, was measured simultaneously for each sample. The PCR efficiency of the reaction was measured with L32 primers using serial dilution of cDNA (1:1, 1:2, 1:4, 1:8 and 1:16). Q-PCR amplification curves were analyzed with iCycler IQ Software version 3.1.7050. For relative quantification of gene expression, the comparative threshold cycle (Ct) method was used (described in User Bulletin 2 for ABI PRISM® 7700 Sequence Detection Systems). The value obtained from Ct represents the PCR cycle at which an increase in fluorescence signal can be detected above background for the particular gene. The Ct values of endogenous control (L32) were subtracted from that of each gene of interest Ct values to derive the ΔCt value. The relative expression of the gene of interest, ΔΔCt, was then evaluated by subtracting the ΔCt of control sample from the compared sample, e.g., male (control) to female (compared) or young (control) to old (compared). The fold difference was calculated as $2^{-\Delta\Delta Ct}$.

RESULTS

Gene Expression in Human Parotid Glands Measured by Array

RNA was isolated from the parotid glands of 8 female and 5 male subjects 19 to 71 years of age and analyzed by microarray. The Affymetrix® GeneChip® HGU133 Plus 2.0 array contains about 54,000 probe sets representing over 18,400 transcripts and variants, including 14,500 well-characterized human genes. The percent array outliers (19) were in the range of 0.12 to 0.31, and the percent of a single outlier was in the range of 0.11 and 1.02 (19). The percent of “present” call varied from array to array, ranging from 41.2% to 52.5%. About 21% of the probe sets were not detected on any of the 13 arrays. There was a common present call for about 26% of the probe sets on all 13 arrays. An additional 16% of the positive probe sets were detected on at least ten of the 13 arrays. Thus, nearly 37% of the probes sets were “present” on 10 of the 13 samples analyzed, which is similar to the number of genes expressed in other organ systems (25,26).

Age Related Differences in Human Female Parotid Gland Gene Expression

Array data from human female parotid glands were compared for differences in gene expression. Data were analyzed by GeneSifter array data analysis system using RMA (robust multi-array analysis) and filtering criteria of a 1.5 or greater fold change (see METHODS). To eliminate gender differences in gene expression, only female samples were used for this analysis. Samples from 3 young females (19, 25 and 38 years old) and 3 older females (65, 65 and 69 years old) were compared (Figure 1). The expression of 228 probe sets showed differential expression between these two age groups; the signal on 155 (68%) of these probe sets increased in aged parotid glands (Figure 2, Supplement Table A). Probe sets representing 10 unique genes showed a 3 fold or more difference in expression during aging including e.g., CXCL10, UBD, HLA-DQA1 (Table 2, also see full Supplement Table 1). A few examples of

highly differentially expressed genes are indicated by arrows in Figure 2. The differentially expressed genes are involved in numerous biological functions such as chemokine (CXCL10), electron transport (DHRS2), ion transport (SLC10A4), antigen processing (HLA-DQA1), proteolysis (UBD).

Interestingly, a large number of the differentially expressed genes include those known to be involved in immune responses (Table 3). The very high z-score (11.72, Table 3) indicates that the older population may have an altered immune system. Out of the 522 probe sets on the HGU133A Plus 2.0 array known to be associated with the immune system, 30 probe sets were differentially expressed. The expression decreased on 22 probe sets while expression increased on 8 probe sets in the older population. A list of the 30 differentially expressed probe sets involved in immune response is given in Table 4. The expression of both HLA-DQA1 and HLA-DQB1 are decreased in the parotid gland of the aged female, as well as Chemokine (C-X-C motif) ligand 10 (CXCL10). Several other proteins (e.g., IRF6, IRF7, GBP1, IFITM1, IFITM2, PSMB8 and PSMB9), which are known to be involved in different immune response pathways, showed altered expression in the aged population (Table 4).

The older population also showed altered expression of several ion transporters and neurotransmitter receptors known to be involved in saliva secretion, e.g., the cholinergic muscarinic type 1 receptor CHRM1 and the K channel KCNJ2 showed lower expression in the aged (Supplement Table A). Other ion transporters and channels, such as, SLC10A4, CTHRC1 (Phosphate/organic transporter), SLC21A3, SLC01A2, SLC24A3 SLC30A9, SCL39A10 and CLCN3 also showed differential expression (Supplement Table A) [the data from the 13 individual arrays are accessible for download through the National Center for Biotechnology Information's Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) through series accession number (GSE8764)].

Gender Related Differences in Human Parotid Tissue

To determine whether gender-related gene expression differences exist in the human parotid gland, eight female samples (19, 25, 38, 41, 49, 65, 65 and 69 years of age) and five male samples (42, 59, 62, 70 and 71 years old) were analyzed by microarray. Examination of the array data by GeneSifter software demonstrated that gender has a very significant influence on gene expression in the parotid gland with 787 probe sets showing differential expression. Table 5 presents the gene expression differences between male and female parotid samples. Male tissues showed higher expression with 320 probe sets, while, 467 probe sets were preferentially expressed at a higher level in females. Ten unique gene probe sets were expressed at greater than a 10-fold difference with an additional 79 gene probes were over-expressed at more than a two-fold difference. Not surprisingly, the greatest differences in expression levels (up to 124-fold) were observed in the genes linked to the X and Y chromosomes (Figure 3 and Table 6). Twenty-five probe sets representing genes on the X chromosome were differentially expressed. Of these, 19 were found at higher levels in female glands, while 6 probe sets were slightly higher in male tissue. HBA2 is involved in oxygen transport while XIST regulates X chromosome inactivation (Figure 3). Multiple probe sets for the XIST gene are present on the array which all show much higher expression in females (Figure 3). All 17 of the probe sets for genes on the Y chromosome showed higher expression in male samples and almost no signal was detected in female samples. Representative examples are shown by arrows in Figure 3. These Y chromosome-specific genes are involved in various biological functions such as protein biosynthesis (RPS4Y1), nucleotide binding (DDX3Y), transcription regulation (SMCY, ZFY).

The distribution on chromosomes 1 to 22 of the 652 genes differentially expressed in male and female parotid glands was analyzed, excluding the X and Y sex chromosomes. The number of differentially expressed genes on a given chromosome was directly related to the total number

of predicted genes present on that chromosome (Figure 4). This result suggests that the distribution of differentially expressed genes is randomly dispersed throughout the genome.

The effect of gender on gene expression in the human parotid gland involved a diverse range of biological processes, molecular functions and cellular components. The z-score analysis indicates the involvement of these genes in several important pathways. As shown in Table 7, gender influenced many genes that affect metabolism, transcription, DNA binding, metal binding and secretory pathway, and they are localized in different cell compartments. Among the differentially expressed genes, several are involved in transcription regulation; e.g., PNN, ASXL1 and ZNF432 are more highly expressed in females, whereas SMCY, ZFY and BHLHB2 are more highly expressed in male parotid tissue (Table 6). Male parotid glands highly expressed submaxillary gland androgen regulated protein B (SMR3A, ont = secretion) and apolipoprotein D (APOD, ont = lipid metabolism); whereas, genes highly expressed in female glands were SPDI, transmembrane family member 2 (SID2, ont = lipid metabolism) and cytochrome B5 reductase (CYB5R3, ont = electron transport) (see GEO # GSE8764). (Supplement Table B).

The Affymetrix® GeneChip® HGU133 Plus 2.0 array contains about 250 probe sets related to the secretory pathway, 16 of which were differentially expressed by gender with a z-score of 3.69 (Table 7). Several proteins involved in exocytosis were found to be differentially expressed in parotid tissue, e.g., members of the SNARE complex such as Syntaxin, VAMP, SNAP and proteins involved in the regulation and formation of the SNARE complex, e.g., RAB (Table 8). ARF3, which encodes for a small guanine nucleotide-binding protein that plays a role in vascular trafficking and as an activator of phospholipase D, also showed differential expression. The GTP binding protein SARA showed higher expression in female parotid tissue. Several members of calcium signaling pathways were also differentially expressed, e.g. CAMK2G, Inositol 3 phosphate 3 kinase B (ITPKB), nitric oxide synthase 3 (NOS3), and the plasma membrane calcium ATPase type 2 (PMCA2) (Table 9).

Validation of Array Data

Gender Related Differences—In addition to the RNA samples analyzed by microarray, RNA samples from another 19 subjects were also isolated for Q-PCR analysis to validate the gender-specific array results. This second group included 11 female subjects (27, 35, 36, 40, 43, 49, 53, 61, 66, 74 and 83 years of age) and 8 males (40, 46, 51, 55, 67, 70, 73 and 85 years old). Four genes which were differentially expressed in human parotid glands were selected for Q-PCR evaluation. Genes highly expressed in male glands included DDX3Y, ZFY and CYTORF15, whereas a gene highly expressed in female glands was XIST. Q-PCR results confirmed the array data (Table 10). Each of the genes tested by Q-PCR showed the same pattern of expression as measured by array technique.

Age Related Differences—To verify the differential gene expression in the young and old female populations as observed by microarray analysis, three genes were selected for Q-PCR (STAT1, CXCL10 and HLA-DQA1). The female parotid from 8 young (19, 27, 35, 36, 38, 40, 43 and 49 years old) and 8 older (53, 61, 65, 65, 66, 69, 74 and 83 years) subjects were used for this analysis. Table 10 shows that the results of Q-PCR study gave the same gene expression pattern as that obtained by microarray. Three genes (SLC10A4, CTHRC1 and CHRM1) were further tested with mixed gender populations of young (19, 27, 36, 38 and 49 year old females; and 40, 46 and 51 year old males) and old (61, 66, 74 and 83 year old females; and 67, 70, 73 and 85 year old males) subjects. As shown in Table 10, all three genes selected for validation by Q-PCR technique further confirmed the array results.

DISCUSSION

Although well documented in rodents, this is the first comprehensive report to demonstrate the inherent gender-specific differences in gene expression in human parotid gland. Our findings are consistent with the gender associated and gland specific variations in mRNA levels previously reported in rodent model salivary gland systems (20,27,28) and in lacrimal (29) and meibomian glands (30). The differentially expressed genes in these studies are involved in a wide range of biological processes, molecular functions and cellular components, including growth and development, transcription, metabolism, signal transduction, ion transport, receptor activity and protein and nucleic acid binding. In the present study, gender-specific differences in expression were noted for 787 out of 54,000 probe sets on the HG U133 Plus 2.0 array, with the majority of these genes being expressed to a higher extend in females (~59%). The proteins encoded by these genes are located in different cell compartments, i.e. the nucleus, plasma membrane, mitochondria and cytoplasm. At this time, we can only speculate as to the biological and physiological implications of the observed sexual dimorphism in salivary gland gene expression. It should be noted that the sexual dimorphism in mice is in part due to gland-specific differences in gene expression between males and females (20), consistent with the gender-related differences in human salivary glands being due to tissue-specific variations in gene expression.

Gender-specific differential gene expression was detected on all chromosomes and the number of differences was found to be directly related to the size of the chromosome, i.e. the larger the chromosome, the greater the number of differences in gene expression that were detected (a similar chromosomal distribution was detected for aging, not shown) suggesting that the distribution of differentially expressed genes is randomly dispersed throughout the genome. The differential gene expression pattern on the X and Y chromosomes of several genes (e.g. UTX, DDX3X, SMCX) are in agreement with previous reports on human lympholastoma cell line (31) and 11 different human tissues (32). The gene ontologies analysis of gender-specific, differential expression patterns provides several examples of genes that potentially explain how gender modulates salivation by the human parotid gland (1,4,15).

Age related differences in gene expression have been noted in the mouse submandibular gland model (33). Using cDNA array analysis, Hiratsuka et al. found that 160 of the 1328 genes screened showed more than a two-fold change, 96% of which exhibited decreased expression in elderly mice (33). These genes are associated with numerous biological pathways, e.g., transcription regulation, ion transport, and signal transduction. The effects of age on specific gene ontologies in the human parotid gland may provide insight into functional and morphological changes previously described (2,3,16).

We also found that age had a significant influence on the expression of genes associated with primary metabolism and physiological processes. These observations were not unexpected considering that aged animals demonstrate a reduced protein biosynthesis, but importantly, our results provide novel information defining which specific genes may be most affected by aging. Of particular interest are those associated with defense/immune responses. The expression of both HLA-DQA1 and HLA-DQB1 are decreased in the parotid gland of the aged. HLA-DQA1 and HLA-DQB1 belong to the histocompatibility complex loci (HLA) class II. The class II molecule is a heterodimer consisting of an alpha (DQA) and beta (DQB) chain, both anchored in the membrane. HLA II plays a central role in the immune system by T-cell activation (34, 35). Chemokine (C-X-C motif) ligand 10 (CXCL10) also showed lower expression in the aged population. Chemokines are a group of low molecular weight peptides that induce the chemotaxis of different leukocyte subtypes. At present, more than 50 chemokines have been described. CXC chemokines attract neutrophils and promote their adherence to endothelial cells. Several other proteins known to be involved in different immune response pathways

showed altered expression in aged population (e.g., IRF1, IRF7, GBP1, IFITM1, IFITM2, IFITM3, PSMB8 and PSMB9). Complex remodeling of the immune system occurs during aging, which may contribute significantly to morbidity and mortality in the elderly (36,37). Despite the great number of studies on changes in the immune system of the elderly, the biological basis of such changes is unclear. This is at least partly due to the alterations observed in the immune system of the elderly that could be a cause or the consequence of the underlying pathological processes. Undoubtedly, diseases such as infectious, autoimmune and neoplastic pathologies, which aged people are particularly susceptible to, involve dysregulation of immune function (36,37). On the other hand, recent studies in healthy centenarians suggest that the immunological changes observed during aging are consistent with a reshaping, rather than a generalized deterioration, of the main immune functions (38).

The number of elderly is dramatically increasing, and consequently, geriatric pathology is becoming a more important aspect of clinical practice. Therefore, it is particularly important to evaluate further the findings in the immune system of the elderly so as to better understand their susceptibility to certain diseases, and the links between health and longevity. Salivary gland function may prove to be a parameter worth evaluating in the aged, as shown in other clinical populations (39,40).

This study is an initial important step in identifying the genes which are differentially expressed due to gender and aging. It is noteworthy that very little overlap was observed between the gender-related and age-related differences in gene expression (<1.3%), indicating that these differences are specific. Our results will hopefully stimulate additional studies in this area, especially clinical studies that aid in the development of strategies to reverse or lessen the negative impact of age-related changes in gene expression on oral health. Although the amount of data obtained from microarray can be overwhelming, informatics tools are emerging that take high quality datasets and permit systems level analysis that can identify key biological pathways and genes that are involved in normal physiological and pathogenic processes (41). Q-PCR analysis confirmed the results of the microarray study, and verified reproducibility of our results in additional independent samples, indicating that glands from another population of subjects of the same age range and/or gender group would very likely generate the same results. Therefore, our results provide critical information for understanding the complex changes in gene expression that may significantly contribute to gender-associated and age-related differences in the secretion mechanism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

REFERENCES

1. Percival RS, Challacombe SJ, Marsh PD. Flow rates of resting whole and stimulated parotid saliva in relation to age and gender. J Dent Res 1994;73(8):1416–1420. [PubMed: 8083437]
2. Yeh CK, Johnson DA, Dodds MW. Impact of aging on human salivary gland function: a community-based study. Aging (Milano) 1998;10(5):421–428. [PubMed: 9932146]
3. Eliasson L, Birkhed D, Osterberg T, Carlen A. Minor salivary gland secretion rates and immunoglobulin A in adults and the elderly. Eur J Oral Sci 2006;114(6):494–499. [PubMed: 17184231]
4. Heintze U, Birkhed D, Bjorn H. Secretion rate and buffer effect of resting and stimulated whole saliva as a function of age and sex. Swed Dent J 1983;7(6):227–238. [PubMed: 6583873]
5. Pedersen W, Schubert M, Izutsu K, Mersai T, Truelove E. Age-dependent decreases in human submandibular gland flow rates as measured under resting and post-stimulation conditions. J Dent Res 1985;64(5):822–825. [PubMed: 3858303]

6. Tylenda CA, Ship JA, Fox PC, Baum BJ. Evaluation of submandibular salivary flow rate in different age groups. J Dent Res 1988;67(9):1225–1228. [PubMed: 3166006]
7. Wu AJ, Baum BJ, Ship JA. Extended stimulated parotid and submandibular secretion in a healthy young and old population. J Gerontol A Biol Sci Med Sci 1995;50A(1):M45–M48. [PubMed: 7814788]
8. Fischer D, Ship JA. Effect of age on variability of parotid salivary gland flow rates over time. Age Ageing 1999;28(6):557–561. [PubMed: 10604508]
9. Elishoov H, Wolff A, Volovikov A, Gorsky M. [Evaluation of unstimulated and stimulated parotid salivary flow rate in Israeli healthy subjects aged 60 years and older]. Refuat Hapeh Vehashinayim 2005;22(2):44–48. 86. [PubMed: 16121957]
10. Cook, D.; Van Lennep, EW.; Roberts, ML.; Young, JA. Secretion by the Major Salivary Glands. In: Johnson, LR., editor. Physiology of the Gastrointestinal Tract. New York: Raven Press; 1994. p. 1061-1117.
11. Turner RJ, Sugiya H. Understanding salivary fluid and protein secretion. Oral Dis 2002;8(1):3–11. [PubMed: 11936453]
12. Guo T, Rudnick PA, Wang W, Lee CS, Devoe DL, Balgley BM. Characterization of the human salivary proteome by capillary isoelectric focusing/nanoreversed-phase liquid chromatography coupled with ESI-tandem MS. J Proteome Res 2006;5(6):1469–1478. [PubMed: 16739998]
13. Melvin JE, Yule D, Shuttleworth T, Begenisich T. Regulation of fluid and electrolyte secretion in salivary gland acinar cells. Annu Rev Physiol 2005;67:445–469. [PubMed: 15709965]
14. Ship JA. Diagnosing, managing, and preventing salivary gland disorders. Oral Dis 2002;8(2):77–89. [PubMed: 11991308]
15. Inoue H, Ono K, Masuda W, Morimoto Y, Tanaka T, Yokota M, et al. Gender difference in unstimulated whole saliva flow rate and salivary gland sizes. Arch Oral Biol 2006;51(12):1055–1060. [PubMed: 16919593]
16. Vissink A, Spijkervet FK, Van Nieuw Amerongen A. Aging and saliva: a review of the literature. Spec Care Dentist 1996;16(3):95–103. [PubMed: 9084322]
17. Brown DA, Bruce JI, Straub SV, Yule DI. cAMP potentiates ATP-evoked calcium signaling in human parotid acinar cells. J Biol Chem 2004;279(38):39485–39494. [PubMed: 15262999]
18. Nakamoto T, Srivastava A, Romanenko VG, Ovitt CE, Perez-Cornejo P, Arreola J, et al. Functional and molecular characterization of the fluid secretion mechanism in human parotid acinar cells. Am J Physiol Regul Integr Comp Physiol 2007;292(6):R2380–R2390. [PubMed: 17347411]
19. Li C, Wong WH. Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. Proc Natl Acad Sci U S A 2001;98(1):31–36. [PubMed: 11134512]
20. Treister NS, Richards SM, Lombardi MJ, Rowley P, Jensen RV, Sullivan DA. Sex-related differences in gene expression in salivary glands of BALB/c mice. J Dent Res 2005;84(2):160–165. [PubMed: 15668334]
21. Reiner A, Yekutieli D, Benjamini Y. Identifying differentially expressed genes using false discovery rate controlling procedures. Bioinformatics 2003;19(3):368–375. [PubMed: 12584122]
22. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 2000;25(1):25–29. [PubMed: 10802651]
23. Kanehisa M, Goto S, Kawashima S, Okuno Y, Hattori M. The KEGG resource for deciphering the genome. Nucleic Acids Res 2004;32(Database issue):D277–D280. [PubMed: 14681412]
24. Doniger SW, Salomonis N, Dahlquist KD, Vranizan K, Lawlor SC, Conklin BR. MAPPFinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. Genome Biol 2003;4(1):R7. [PubMed: 12540299]
25. Wang J, Robinson JF, Khan HM, Carter DE, McKinney J, Miskie BA, et al. Optimizing RNA extraction yield from whole blood for microarray gene expression analysis. Clin Biochem 2004;37 (9):741–744. [PubMed: 15329310]
26. Thach DC, Shaffer KM, Ma W, Stenger DA. Assessing the feasibility of using neural precursor cells and peripheral blood mononuclear cells for detection of bioactive Sindbis virus. Biosens Bioelectron 2003;18(8):1065–1072. [PubMed: 12782470]

27. Murphy RA, Watson AY, Metz J, Forssmann WG. The mouse submandibular gland: an exocrine organ for growth factors. J Histochem Cytochem 1980;28(8):890–902. [PubMed: 6969274]
28. Senorale-Pose M, Jacqueson A, Rougeon F, Rosinski-Chupin I. Acinar cells are target cells for androgens in mouse submandibular glands. J Histochem Cytochem 1998;46(5):669–678. [PubMed: 9562575]
29. Richards SM, Jensen RV, Liu M, Sullivan BD, Lombardi MJ, Rowley P, et al. Influence of sex on gene expression in the mouse lacrimal gland. Exp Eye Res 2006;82(1):13–23. [PubMed: 15979613]
30. Richards SM, Yamagami H, Schirra F, Suzuki T, Jensen RV, Sullivan DA. Sex-related effect on gene expression in the mouse meibomian gland. Curr Eye Res 2006;31(2):119–128. [PubMed: 16500762]
31. McRae AF, Matigian NA, Vadlamudi L, Mulley JC, Mowry B, Martin NG, et al. Replicated effects of sex and genotype on gene expression in human lymphoblastoid cell lines. Hum Mol Genet 2007;16 (4):364–373. [PubMed: 17164263]
32. Talebizadeh Z, Simon SD, Butler MG. X chromosome gene expression in human tissues: male and female comparisons. Genomics 2006;88(6):675–681. [PubMed: 16949791]
33. Hiratsuka K, Kamino Y, Nagata T, Takahashi Y, Asai S, Ishikawa K, et al. Microarray analysis of gene expression changes in aging in mouse submandibular gland. J Dent Res 2002;81(10):679–682. [PubMed: 12351665]
34. Zang W, Murphy B. Peptide-mediated immunosuppression. Am J Ther 2005;12(6):592–599. [PubMed: 16280653]
35. O'Hanlon TP, Carrick DM, Targoff IN, Arnett FC, Reveille JD, Carrington M, et al. Immunogenetic risk and protective factors for the idiopathic inflammatory myopathies: distinct HLA-A, -B, -Cw, -DRB1, and -DQA1 allelic profiles distinguish European American patients with different myositis autoantibodies. Medicine (Baltimore) 2006;85(2):111–127. [PubMed: 16609350]
36. Katz JM, Plowden J, Renshaw-Hoelscher M, Lu X, Tumpey TM, Sambhara S. Immunity to influenza: the challenges of protecting an aging population. Immunol Res 2004;29(1–3):113–124. [PubMed: 15181275]
37. Vesosky B, Turner J. The influence of age on immunity to infection with Mycobacterium tuberculosis. Immunol Rev 2005;205:229–243. [PubMed: 15882357]
38. Franceschi C, Bonafè M. Centenarians as a model for healthy aging. Biochem Soc Trans 2003;31:457–461. [PubMed: 12653662]
39. Nieuw Amerongen AV, Ligtenberg AJ, Veerman EC. Implications for diagnostics in the biochemistry and physiology of saliva. Ann N Y Acad Sci 2007;1098:1–6. [PubMed: 17303829]
40. Samaranayake L. Saliva as a diagnostic fluid. Int Dent J 2007;57(5):295–299. [PubMed: 17992912]
41. Langfelder P, Horvath S. Eigengene networks for studying the relationships between co-expression modules. BMC Syst Biol 2007;1(1):54. [PubMed: 18031580]

ACKNOWLEDGEMENTS

We thank Zugen Chen, Jason Liu and Shilpa Patel at UCLA microarray core facility for their cooperation in generating the microarray data. We also thank Eastern, Mid Western and South Division of the Cooperative Human Tissue Network for the supply of the human salivary gland tissue. This research was supported in part by PHS grants RO1-DE09692, R37-DE08921 (JEM), T32-DE07202 (AS), RO1-DE17593 (DTW) and T32-DE07296 (JW).

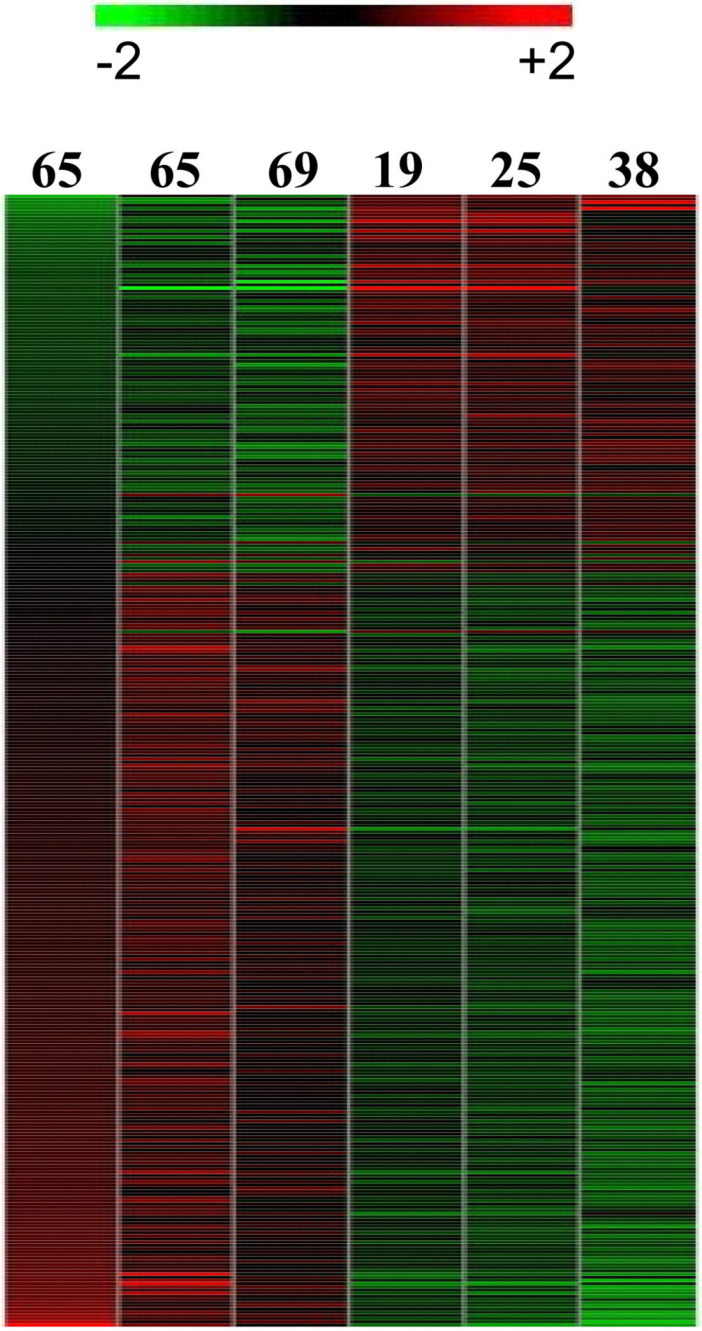

Figure 1.
Heat map of 228 differentially expressed genes comparing RNA samples from female human parotid of different age groups. The age is noted on each lane. Red represents relative expression greater than the median expression level across all samples, and green represents an expression level lower than the median. Black indicates intermediate expression.

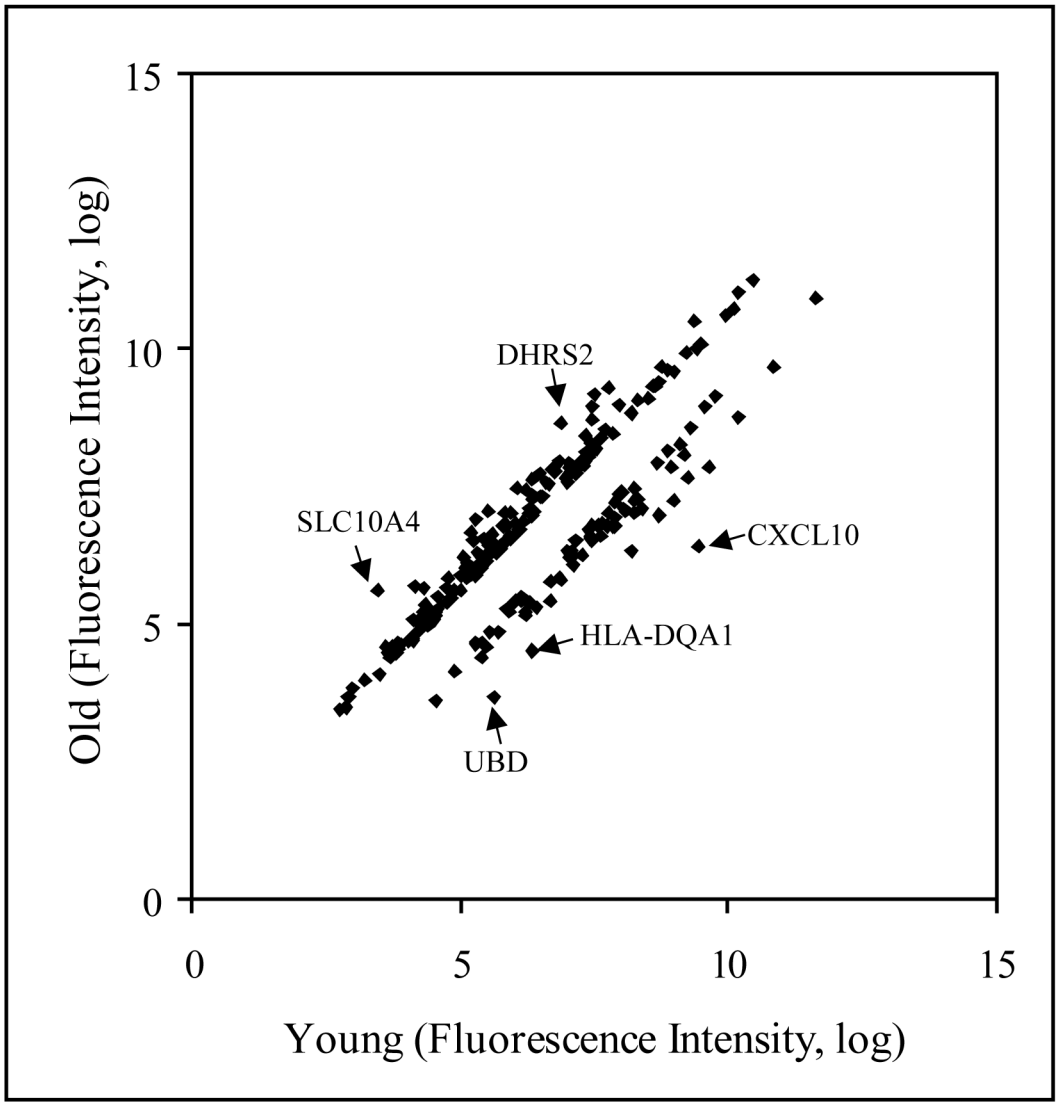

Figure 2.
Scattered plot analysis of 228 genes, which have at least 1.5 fold difference in their expression in between young (19, 25 and 38 years of age) and old (65, 65 and 69 years of age) female parotid glands.

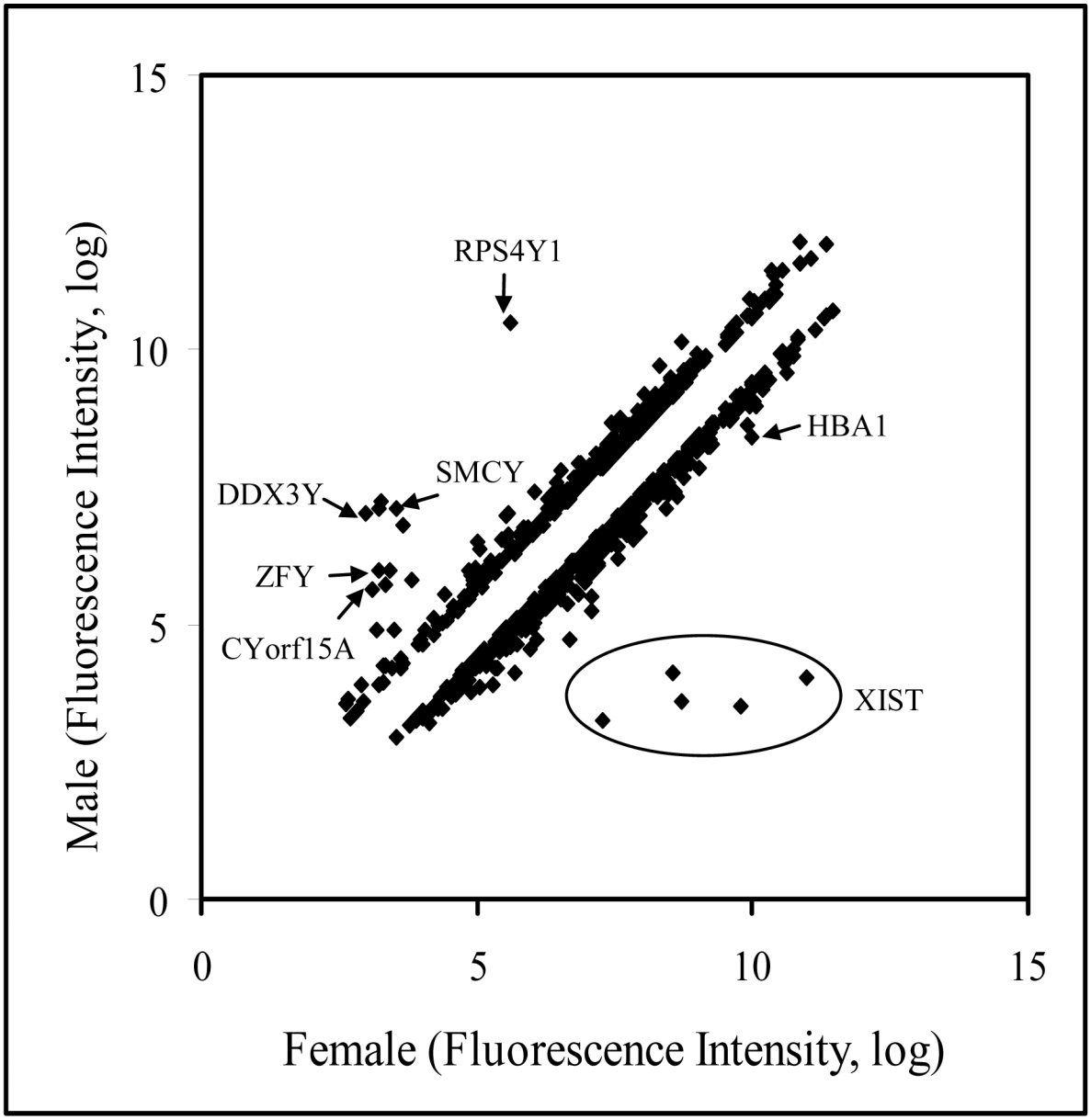

Figure 3.
Scattered plot analysis of 787 genes, which have at least 1.5 fold differences in their expression in between male and female parotid glands.

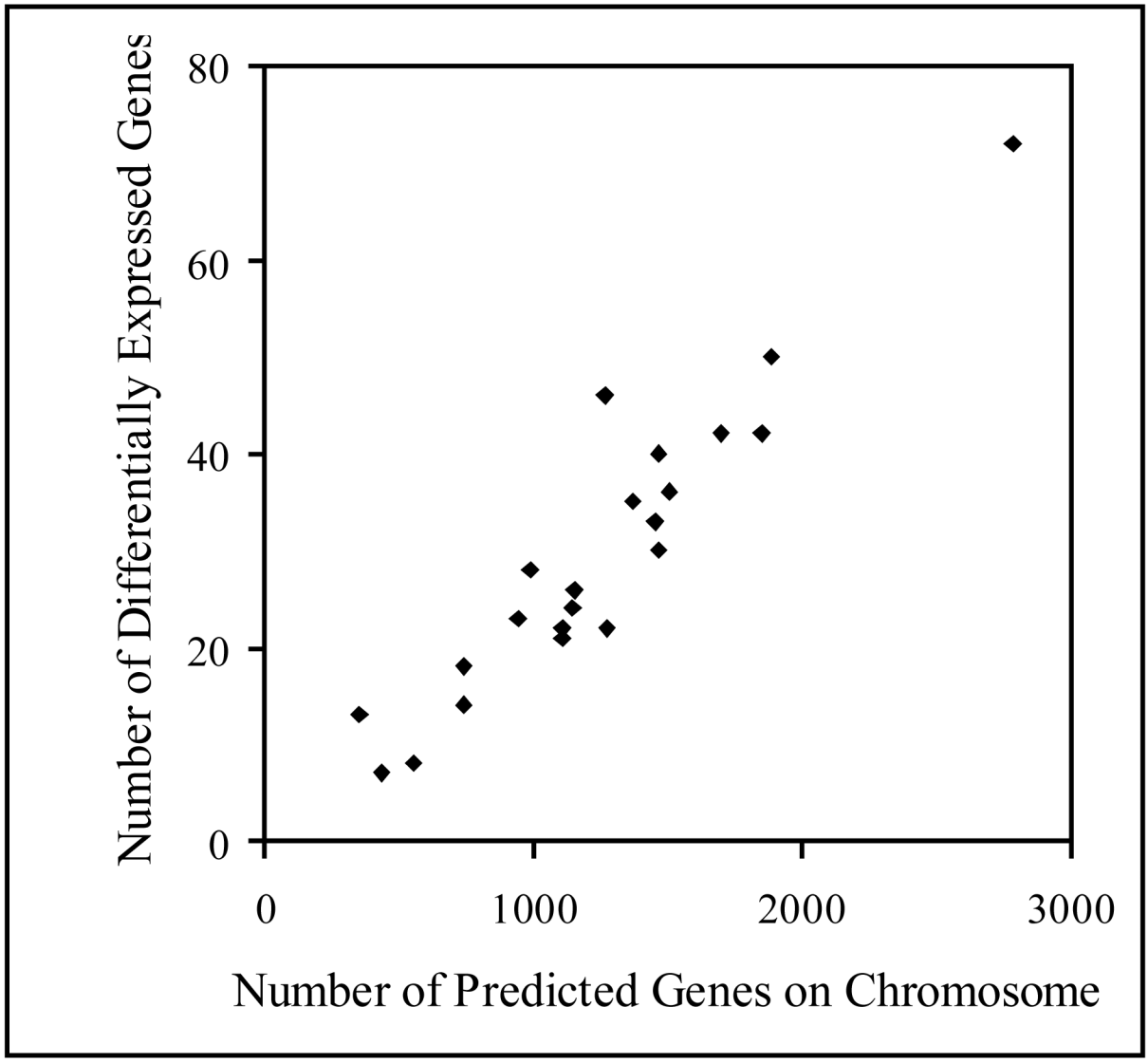

Figure 4.
A direct correlation in between number of genes on a chromosome and the number of differentially expressed genes on that chromosome

Table 1

Oligonucleotide primers used for Q-PCR

Accession No.	Gene ID	Forward (5'–3')	Reverse (5'–3')
NM_000738	CHRM1	TTCCTGGGAGTGGGAGTCAAG	ATTGGGGAGCTCACAGGAGAG
NM_138455	CTHRC1	TCGCACTTCTTCTGTGGAAGG	TGCGAGAAACTGAATTCCATCC
AF332225	CYORF15B	GGCAGTTTCTTAGGCTGTGAC	TTGTTTCCAATGCTAGCCAGAG
NM_004660	DDX3Y	ACTGATAGGAAGGTCCACATCC	AATACTGCTGGCTGGTAAAACC
NM_152679	SLC10A4	TGTGGAGATACACAGGAGCTTC	GGCTTCACGTTAGCCATTCC
NM_139266	STAT1	CAAACCTCAAGCCAGCCTTG	GGCAGTAACACGGGGATCTC
NR_001564	XIST	AAACAAGGTGTTGTGGTCTTCC	TCAGCTGTCAGTGATCTAATGC
BC033974	ZFY	CTTCCCTCTCACTCCTGGTAC	CAGGCAGAAGAAAGAATCAGCA
NM_001565	CXCL10	GAGGTGCTATGTTCTTAGTGGATG	CTGAAAGAATTTGGGCCCCTTG
NM_002122	HLA-DQA1	GCTATATCCCCTCAGAGCTCAC	AGTCAGCCCTGGATGAAAGATG

Table 2

Gene expression ratio of older (65, 65 and 69 years) to younger (19, 25 and 38 years) (O/Y) female parotid glands*

Gene Name	Acc. No.	Gene ID	Ratio (O/Y)	Direction	Ontology
Cytokine subfamily B (Cys-X-Cys), member 10	NM_001565	CXCL10	8.37	Down	Chemokine
Solute carrier family 10 member 4	AI421796	SLC10A4	4.41	Up	Ion transport
Diubiquitin D	NM_006398	UBD	3.89	Down	Proteolysis
Histocompatibility complex, class II, DQ alpha 1	BG397856	HLA-DQA1	3.5	Down	Antigen processing
Interferon, alpha-inducible protein	NM_022873	IFI6	3.49	Down	Release of cytochrome c from mitochondria
Short-chain alcohol dehydrogenase family member	NM_005794	DHRS2	3.38	Up	Electron transport
Interferon-stimulated transcription factor 3, gamma	NM_006084	ISGF3G	3.34	Down	Regulation of transcription
Signal transducer and activator of transcription 1, 91kDa	BC002704	STAT1	3.31	Down	Regulation of transcription
Interferon-stimulated protein, 15 kDa	NM_005101	ISG15	3.05	Down	Response to other organism
Ependymin related protein 1	BC000686	EPDR1	3	Up	Ion binding
Cysteine/tyrosine-rich 1	H06649	CYYR1	2.92	Up	
Periostin, osteoblast specific factor	D13665	POSTN	2.86	Up	Skeletal development
Collagen triple helix repeat containing 1	AA584310	CTHRC1	2.82	Up	Phosphate transport
Histone deacetylase 9	BM726008	HDAC9	2.73	Up	Negative regulation of transcription
Phosphodiesterase 5A, cGMP-specific	BF221547	PDE5A	2.68	Up	Signal transduction
Immunoglobulin heavy constant gamma 1	BC001872	IGHG1	2.66	Down	Antigen processing
Stearoyl-CoA desaturase	AA678241	SCD	2.49	Down	Lipid metabolic process
Rho GTPase activating protein 6	NM_001174	ARHGAP6	2.4	Up	Regulation of catalytic activity
Glycoprotein (transmembrane) nmb (GPNMB)	NM_002510	GPNMB	2.37	Up	Negative regulation of cellular process
Interferon regulatory factor 7	NM_004030	IRF7	2.36	Down	Negative regulation of transcription
Four and a half LIM domains 1	AF063002	FHL1	2.31	Up	Metal Binding
Lysosomal associated protein transmembrane 4 beta	AW149681	LAPTM4B	2.31	Up	Transport
Cytochrome P450, family 4, subfamily B, polypeptide 1	J02871	CYP4B1	2.26	Up	Electron transport
Frizzled-related protein	U91903	FRZB	2.23	Up	Cell communication
Four and a half LIM domains 1 (FHL1)	NM_001449	FHL1	2.18	Up	Metal Binding
Solute carrier family 1 , member 1	AW235061	SLC1A1	2.16	Up	Transport
Epithelial stromal interaction 1	AA781795	EPSTI1	2.15	Down	
Guanylate binding protein 1, interferon-inducible	AW014593	GBP1	2.15	Down	Immune response
Tubulin tyrosine ligase-like family, member 7	NM_024686	TTLL7	2.14	Up	Protein modification

Gene Name	Acc. No.	Gene ID	Ratio (O/Y)	Direction	Ontology
NGFRAP1-like 1	AV726956	NGFRAP1L1	2.13	Down	
Fatty acid synthase	AI954041	FASN	2.12	Down	Fatty acid biosynthetic process
Guanylate binding protein 1, interferon-inducible	BC002666	GBP1	2.1	Down	Immune response
Interferon induced transmembrane protein 3 (1-8U)	BF338947	IFITM3	2.1	Down	Immune response
Asparaginase like 1	NM_025080	ASRGL1	2.09	Up	Glycoprotein catabolic process
Myxovirus (influenza virus) resistance 2	NM_002463	MX2	2.09	Down	Defense response
Solute carrier organic anion transporter, member 1A2	NM_021094	SLCO1A2	2.09	Up	Transport
Interferon induced transmembrane protein 1	AA749101	IFITM1	2.08	Down	Immune response
Plasminogen activator, tissue	NM_000930	PLAT	2.07	Up	Protein modification
Solute carrier family 25, member 34	AU151211	SLC25A34	2.07	Up	Transport
ATP-binding cassette, member 2 (ABCB2)	NM_000593	TAP1	2.03	Down	Response to stimulus
Low density lipoprotein receptor	NM_000527	LDLR	2.03	Down	Protein Biosysnthesis
Cysteine/tyrosine-rich 1	AI458003	CYYR1	2.02	Up	
NLR family, CARD domain containing 5	AA005023	NLRC5	2.02	Down	Defense response
Lysosomal associated protein transmembrane 4 beta	NM_018407	LAPTM4B	2.01	Up	Transport
Monocyte to macrophage differentiation-associated	NM_012329	MMD	2.01	Up	Cytolysis
Peroxisomal biogenesis factor 6	NM_000287	PEX6	2.01	Down	Peroxisome organization
Integrin beta 1 binding protein 1	NM_004763	ITGB1BP1	2	Up	Cell adhesion

*Genes listed had signal intensity of >5.0 in at least one group, expression ratio of >2.0 (between glands), p value <0.05, with known gene identity.

Table 3

Differential gene expression in older (69,65 and 65 years) compared to younger (19, 25 and 38) female parotid glands and their ontological categorization based on their immunity response

Ontology	Diff exp genes[a]	Up-reg[b]	Down-reg[b]	Tot on array[c]	z-Score up[d]	z-Score down[d]
Immune response	28	4	24	522	−1.14	11.72
Defense response	16	6	10	476	−0.13	4.14
Antigen processing and presentation	9	0	9	52	−0.84	15.24
Response to biotic stimulus	9	0	9	215	−1.37	8.58
Response to other organism	7	1	6	151	−0.72	5.23
Response to virus	6	0	6	84	−1.07	7.57

[a] Diff exp genes indicates the total number of genes differentially expressed on the array in that category.

[b] Up-reg and down-reg indicate the total number of up and down regulated genes respectively in older population.

[c] Tot on array indicates the total number of genes on the array in that onctological category.

[d] z-score-up and z-score-down indictae the z-score for that category.

Table 4

Differentially expressed genes known to be involved in the immune response in older (65, 65 and 69 years) compared to younger (19, 25 and 38 years) female parotid glands

Gene Name	Accession No.	Gene ID	Ratio	Direction
Chemokine (C-X-C motif) ligand 10	NM_001565	CXCL10	8.37	Down
Ubiquitin D	NM_006398	UBD	3.89	Down
Major histocompatibility complex, class II, DQ alpha 1	BG397856	HLA-DQA1	3.5	Down
Interferon, alpha-inducible protein 6	NM_022873	IFI6	3.49	Down
Interferon-stimulated transcription factor 3, gamma 48kDa	NM_006084	ISGF3G	3.34	Down
interferon-stimulated protein, 15 kDa	NM_005101	ISG15	3.05	Down
Histone deacetylase 9	BM726008	HDAC9	2.73	Up
interferon regulatory factor 7	NM_004030	IRF-7	2.36	Down
guanylate binding protein 1, interferon-inducible, 67kD	AW014593	GBP1	2.15	Down
Guanylate binding protein 1, interferon-inducible	BC002666	GBP1	2.1	Down
Interferon induced transmembrane protein 3	BF338947	IFITM3	2.1	Down
Myxovirus (influenza virus) resistance 2	NM_002463	MX2	2.09	Down
Interferon induced transmembrane protein 1	AA749101	IFITM1	2.08	Down
Transporter 1, ATP-binding cassette, sub-family B	NM_000593	TAP1	2.03	Down
Major histocompatibility complex, class II, DQ beta 1	AI583173	HLA-DQB1	1.99	Down
Myeloid leukemia factor 1	NM_022443	MLF1	1.97	Up
Proteasome subunit, beta type, 9	NM_002800	PSMB9	1.97	Down
NCK adaptor protein 1	NM_006153	NCK1	1.95	Up
Suppressor of cytokine signaling 5	AW664421	SOCS5	1.95	Up
Interferon regulatory factor 1	NM_002198	IRF-1	1.89	Down
Secreted and transmembrane 1	BF939675	SECTM1	1.82	Down
Leptin	NM_000230	LEP	1.74	Up
Clusterin	M25915	CLU	1.67	Up
CD74 molecule	K01144	CD74	1.63	Down
Mucosa associated lymphoid tissue lymphoma translocation ge	NM_006785	MALT1	1.63	Up
Proteasome subunit, beta type, 9	AI375915	PSMB9	1.62	Down
Proteasome subunit, beta type, 8	U17496	PSMB8	1.61	Down
Tumor necrosis factor (ligand) superfamily, member 13b	AF134715	TNFSF13B	1.58	Down
Interferon induced transmembrane protein 2	NM_006435	IFITM2	1.55	Down
Chemokine (C-C motif) receptor 2	NM_000647	CCR2	1.5	Up

Table 5

Differential expression of probe sets in male (n=5) and female (n=8) parotid glands*

Expression Threshhold	Differentially Expressed Genes	Genes Up-reguatted in Male	Genes Up-regulated in Female
>1.5 fold	787	320	467
>1.5 fold and <2	698	280	418
>2 fold and <10	79	35	44
>10 fold	10	5	5

* A student t-test was applied, with the p value <0.05.

Table 6

Gender related differential gene expression in human parotid gland*

Higher Expression in Female				
Gene Name	**Accession No.**	**Gene ID**	**Ratio**	**Ontology**
X (inactive)-specific transcript	NR_001564	XIST	124.03	
Zinc finger, CCHC domain containing 2	NM_017742	ZCCHC2	3.52	Metal Ion Binding
Pinin, desmosome associated protein	NM_002687	PNN	3.03	Transcription regulation
Hemoglobin, alpha1	NM_000517	HBA1	3.01	Oxygen transport
PRO1073 protein	NM_014086	PRO1073	2.62	Unknown
Serpin peptidase inhibitor clade B (ovalbumin), member 9	BC002538	SERPINB9	2.59	Anti-apoptosis signal transduction
Collagen, type VI, alpha 2	NM_001849	COL6A2	2.53	Organization and biogenesis
Family with sequence similarity 108 member 1	NM_031213	FAM108A1	2.49	Hydrolase activity
WW, C2 and coiled-coil domain containing 1	NM_015238	WWC1	2.46	Cellular function
ADP-ribosylation factor 3	NM_001659	ARF3	2.45	GTPase signal transduction
ATPase, Class V, type 10C	NM_024490	ATP10A	2.45	Cation transport
Immunoglobulin heavy constant mu	BC001872	IGHG1	2.43	Immune response
Immediate early response 3 interacting protein 1	NM_016097	IER3IP1	2.33	Integral to membrane
ATP-binding cassette (CFTR/MRP), member 10	NM_033450	ABCC10	2.3	Transport
Rabaptin, RAB GTPase binding effector protein 2	NM_024816	RABEP2	2.27	Endocytosis, protein transport
Essential meiotic endonuclease 1 homolog 1	NM_152463	EME1	2.27	DNA repair
Misshapen-like kinase 1(zebrafish) (MINK1	NM_015716	MINK1	2.26	Protein Phosphorylation
Arachidonate 5-lipoxygenase	NM_000698	ALOX5	2.25	Linoleic acid metabolism
Secreted phosphoprotein 1	NM_001040058	SPP1	2.18	TGF Beta Signaling Pathway
Small nuclear ribonucleoprotein polypeptide A	NM_004596	SNRPA	2.16	mRNA processing
Member RAS oncogene family	NM_021168	RAB40C	2.15	GTPase signal transduction
RUN and TBC1 domain containing 1	BC029251	RUTBC1	2.13	Unknown
Protease, serine, 21	NM_006799	PRSS21	2.13	Proteolysis
Additional sex combs like 1	NM_015338	ASXL1	2.1	Transcription regulation
Insulin-like growth factor 2 mRNA binding protein 2	NM_006548	IGF2BP2	2.08	Protein biosynthesis
Zinc finger protein 432	NM_014650	ZNF432	2.07	Transcription regulation
Metastasis associated lung adenocarcinoma transcript 1	NR_002819	MALAT1	2.07	Binding
UDP-N-acetyl-alpha-D-galactosamine: Polypeptide N-acetylgalactosaminyltransferase-like 1	AI097463	GALNTL1	2	Glycan biosynthesis

Higher Expression in Male				
Gene Name	**Accession No.**	**Gene ID**	**Ratio**	**Ontology**
Ribosomal protein S4, Y-linked (RPS4Y)	NM_001008	RPS4Y1	29.65	Protein Biosysnthesis
DEADH (Asp-Glu-Ala-AspHis) box polypeptide, Y linked	NM_004660	DDX3Y	16.17	Nucleotide binding
Chromosome Y open reading frame 15A, Testis protein	AF332224	CYorf15A	15.48	
Eukaryotic translation initiation factor 1A, Y-linked	BC005248	EIF1AY	14.74	Protein Biosysnthesis
Smcy homolog, Y-linked	NM_004653	SMCY	11.81	Transcription regulation
Zinc finger protein, Y-linked	NM_003411	ZFY	6.79	Transcription regulation
Ubiquitin specific peptidase 9, Y-linked	NM_004654	USP9Y	5.28	Ubiquitin-dependent protein catabolism
Lumican	NM_002345	LUM	2.82	visual perception, Collagen fibril organization
Cofilin 1(non-muscle)	NM_005507	CFL1	2.63	Signal Transduction
SH3 domain binding glutamic acid-rich protein like	NM_003022	SH3BGRL	2.61	SH3/SH2 adaptor activity
Cysteine-rich secretory protein 2	NM_003296	CRISP2	2.51	Testis Specific
Acid phosphatase, testicular	NM_033068	ACPT	2.43	Riboflavin metabolism
RNA binding motif protein 3	NM_006743	RBM3	2.33	RNA Processing
Rho GTPase activating protein 5	NM_001173	ARHGAP5	2.24	GTPase mediated signal transduction
Inhibin beta A	NM_002192	INHBA	2.22	TGF Beta Signaling Pathway
Short coiled-coil protein	NM_032547	SCOC	2.18	Unknown
Crystallin, beta B2	NM_000496	CRYBB2	2.16	Visual perception
Apolipoprotein D	NM_001647	APOD	2.15	Lipid metabolism
Similar to ubiquitin B precursor	NM_018955	UBB	2.13	Protein ubiquitination
HBS1-like	NM_006620	HBS1L	2.12	Protein Biosynthesis
Replication protein A3	NM_002947	RPA3	2.11	DNA replication
Chromosome 1 open reading frame 43	NM_015449	C1orf43	2.11	Unknown
Cysteine/tyrosine-rich 1	NM_052954	CYYR1	2.1	Unknown
Basic helix-loop-helix domain containing, class B, 2	NM_003670	BHLHB2	2.08	Transcription regulation
Chromosome 1 open reading frame 80	NM_022831	C1orf80	2.06	Unknown
Fatty acid binding protein 7	NM_001446	FABP7	2.05	Fatty acid metabolism
Ras-related C3 botulinum toxin substrate 1	NM_006908	RAC1	2.04	GTPase mediated signal transduction

* Genes listed had a signal intensity of >5.0 in at least one group, expression ratio of >2.0 (between glands), p value <0.05, with known gene identity.

Table 7

Gene expression in male and female human parotid glands and their ontological categorization

Ontology	Diff exp genes[a]	M up[b]	F up[c]	Total on Array[d]	M z-score[e]	F z-score[f]
Biological Processes						
Physiological process	387	176	211	9550	1.54	2.21
Metabolism	275	122	153	6530	0.99	2.25
Regulation of biological process	139	51	88	3375	−1.34	2.53
Protein metabolism	122	64	58	2623	3.01	0.51
Transcription	76	23	53	1977	−2.24	2.04
Cell organization and biogenesis	66	38	28	1405	2.86	−0.26
Protein transport	33	16	17	534	2.22	1.82
Protein biosynthesis	28	17	11	525	2.63	0.01
Vesicle-mediated transport	25	15	10	352	3.62	1.01
Protein kinase cascade	19	10	9	278	2.35	1.36
Response to wounding	19	5	14	363	−0.57	2.4
Macromolecule catabolism	18	11	7	332	2.18	0.03
Secretion	16	12	4	250	3.69	−0.54
Endocytosis	12	6	6	130	2.48	2.03
Cellular Components						
Intracellular	329	139	190	7016	1.78	4.34
Organelle	259	108	151	5757	0.55	2.8
Membrane-bound organelle	231	97	134	5065	0.77	2.62
Cytoplasm	161	82	79	3131	4.01	1.25
Nucleus	155	56	99	3468	−1.04	2.93
Integral to membrane	117	54	63	3457	−1.32	−2.02
Protein complex	78	41	37	1569	2.58	0.33
Mitochondrion	34	21	13	618	3.05	−0.24
Endoplasmic reticulum	31	19	12	579	2.73	−0.29
Organelle membrane	30	15	15	474	2.26	1.38
Extracellular region	28	15	13	998	−0.76	−2.11
Ribonucleoprotein complex	23	18	5	330	5.04	−0.91

Ontology	Diff exp genes[a]	M up[b]	F up[c]	Total on Array[d]	M z-score[e]	F z-score[f]
Envelope	19	10	9	252	2.6	1.44
Molecular Processes						
Protein binding	204	97	107	4621	2.51	0.77
Ion binding	120	41	79	3370	−2.64	0.74
Nucleotide binding	99	39	60	1790	1.61	3.65
Transferase activity	70	22	48	1665	−1.35	2.1
ATP coupled activity and binding	60	22	38	1196	0.33	2.47
Kinase activity	37	11	26	733	−0.47	2.6
Pyrophosphatase activity	33	19	14	502	3.63	0.95
GTP binding	23	14	9	290	4.12	1.08
Transcription factor activity	23	6	17	791	−2.15	−0.07
Structural constituent of ribosome	12	9	3	150	4.06	−0.16
Calmodulin binding	11	3	8	120	0.66	3.37
Hydrogen ion transporter activity	10	4	6	96	1.85	2.74

[a] Diff exp genes indicate the total number of genes differentially expressed on the array in that category.

[b] up regulated genes in male parotid glands, as compared to those of female (M-up).

[c] Up regulated genes in female parotid glands, as compared to those of male (F-up).

[d] Total on array indicates the total number of genes on the array in that Ontological category.

[e] z-score-M and z-score-F indicate the z-score for the male and female respectively.

[f] The z-scores with value >2.0 or <-2.0 are reported for ontologies with more than 10 differentially expressed genes.

Table 8

Differentially expressed genes in male and female human parotid glands known to be involved in secretion

Gene Name	Accession No.	Gene ID	Ratio (M/F)	Direction
ADP-ribosylation factor 3	NM_001659	ARF3	2.45	Down
Calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma	NM_172173	CAMK2G	2.38	Down
Inhibin, beta A	NM_002192	INHBA	2.22	Up
Signal recognition particle 54kDa	NM_003136	SRP54	1.82	Up
Vesicle-associated membrane protein 3 (cellubrevin)	NM_004781	VAMP3	1.82	Up
RAB1A, member RAS oncogene family	NM_004161	RAB1A	1.76	Up
tumor necrosis factor (ligand) superfamily, member 13b	NM_006573	TNFSF13B	1.75	Down
RAB1A, member RAS oncogene family	NM_004161	RAB1A	1.73	Up
Epidermal growth factor receptor pathway substrate 15-like 1	NM_021235	EPS15R	1.66	Down
SEC24 related gene family, member D	NM_014822	SEC24D	1.63	Up
Syntaxin binding protein 3	NM_007269	STXBP3	1.63	Up
Caspase recruitment domain family, member 8	NM_014959	CARD8	1.61	Down
Translocation associated membrane protein 1	NM_014294	TRAM1	1.61	Up
RAB2, member RAS oncogene family	NM_002865	RAB2	1.61	Up
RAB22A, member RAS oncogene family	NM_020673	RAB22A	1.61	Up
Folate receptor 1	NM_016731	FOLR1	1.59	Up
GTP-binding protein Sara	NM_016103	SAR1B	1.59	Up
ATP-binding cassette, sub-family A (ABC1), member 1	NM_005502	ABCA1	1.58	Down
Syntaxin binding protein 1	NM_003165	STXBP1	1.56	Down
Synaptosomal-associated protein, 23kDa	NM_130798	SNAP23	1.53	Up

Table 9

Differentially expressed genes in male and female parotid glands known to be involved in calcium signaling pathways

Gene Name	Accession No.	Gene ID	Ratio (M/F)	direction
Inositol 1,4,5-triphosphate 3-kinase B	NM_002221	IP3K	1.8	down
Nitric Oxide synthase 3	NM_024711	NOS3	1.65	down
Calcium clamoduline-dependent protein kinase (CaM Kinase) II gamma	NM_172170	CAMK2G	2.38	down
ATPase, Ca2+ transporting, plasma membrane	X63575	PMCA2	1.72	down
Voltage-dependent anion channel 3	U90943	VDAC3	1.77	up
Guanine nucleotide binding protein (G protein) q polypeptide	NM_002072	GNAQ	1.98	up

Table 10

Q-PCR confirmation of selected HGU133A 2.0 microarray results

Sex Differences (Sample number used for QPCR, Female n= 11; Male n=8)						
AccessioN No.	**GeneID**	**Fold Changes (M/F)**				**Gene Name**
		Microarray	**p Value**	**Q-PCR**	**p Value**	
NR_001564	XIST	−124.03	0.00	−106.0	0.00	X (inactive)-specific transcript
NM_004660	DDX3Y	16.17	0.00	40.0	0.00	DEADH (Asp-Glu-Ala-AspHis) box polypeptide, Y chromosome
BC033974	ZFY	6.79	0.00	15.0	0.00	Zinc finger protein, Y-linked
AF332225	CYorf15B	15.48	0.00	22.0	0.00	Chromosome Y open reading frame 15B
Differences due to aging (Sample number used for QPCR, Young n = 8; Old n = 8)						
Accession No.	**GeneID**	**Fold Changes (O/Y)**				**Gene Name**
		Microarray	**p Value**	**Q-PCR**	**p Value**	
BC002704	STAT1	−3.3	0.035	−1.51	0.03	Signal transducer and activator of transcription 1
NM_001565	CXCL10	−8.37	0.041	−1.96	0.05	Small inducible cytokine subfamily B (Cys-X-Cys), member 10
NM_002122	HLA-DQA1	−3.5	0.035	−1.57	0.05	Histocompatibility complex, class II, DQ alpha 1
NM_152679	SLC10A4*	4.41	0.032	2.00	0.05	Solute carrier family 10 (sodium/bile acid cotransporter family), member 4
NM_138455	CTHRC1*	2.82	0.047	1.81	0.05	Collagen triple helix repeat containing 1
AI500293	CHRM1*	−1.61	0	−1.28	0.04	Cholinergic receptor, muscarinic 1

* Tested with mixed population of male and female young and old subjects