Wistar rats exposed to bisphenol A in the drinking water. Regul Toxicol Pharmacol 1999; **30**: 130–139.
86. Tyl RW, Myers CB, Marr MC, Thomas BF, Keimowitz AR, Brine DR, Veselica MM, Fail PA, Chang TY, Seely JC, Joiner RL, Butala JH, Dimond SS, Cagen SZ, Shiotsuka RN, Stropp GD, and Waechter JM. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicol Sci 2002; **68**: 121–146.
87. Kawamura Y, Inoue K, Nakazawa H, Yamada T, and Maitani T. Cause of bisphenol A migration from cans for drinks and assessment of inproved cans. Syokuhin Eiseigaku Zassi 2001; **42**: 13–17.
88. Yoshida M, Shimomoto T, Katashima S, Watanabe G, Taya K, and Maekawa A. Maternal exposure to low doses of bisphenol A has no effects on development of female reproductive tract and uterine carcinogenesis in Donryu rats. J Reprod Dev 2004; **50**: 349–360.
89. Takahashi O and Oishi S. Disposition of orally administered 2,2-bis(4-hydroxyphenol)propane (bisphenol A) in pregnant rats and the placental transfer to fetuses. Environ Health Perspect 2000; **108**: 931–935.
90. Johnson MD, Kenney N, Stoica A, Hilakivi-Clarke L, Singh B, Chepko G, Clarke R, Sholler P, Lirio AA, Foss C, Reiter R, Trock B, Paik S, and Martin MB. Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. Nature Med 2003; **9**: 1081–1084.
91. Takasugi N. Cytological basis for permanent vaginal changes in mice treated neonatally with steroid hormones. Int Rev Cytol 1976; **44**: 193–224.
92. Newbold RR and Liehr JG. Induction of uterine adenocarcinoma in CD-1 mice by catechol estrogens. Cancer Res 2000; **60**: 235–237.
93. Okada A, Ohta Y, Buchanan DL, Sato T, Inoue S, Hiroi H, Muramatsu M, and Iguchi T. Changes in ontogenic expression of estrogen receptor alpha and not of estrogen receptor beta in the female rat reproductive tract. J Molecular Endocrinol 2002; **28**: 87–97.
94. Watanabe H, Suzuki A, Mizutani T, Khono S, Lubahn DB, Handa H, and Iguchi T. Genome-wide analysis of changes in early gene expression induced by estrogen. Genes to Cells 2002; **7**: 497–507.
95. Watanabe H and Iguchi T. Evaluation of endocrine disruptors based on gene espression using a DNA microarray. Environ Sci 2003; **10**: 061–067.

Mutations in the fibroblast growth factor 10 gene are associated with aplasia of lacrimal- and salivary glands (ALSG)

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Autosomal dominant aplasia of lacrimal- and salivary glands (ALSG; absence of salivary glands [OMIM 180920], congenital alacrima [OMIM103420]) is a rare condition[1] characterized by irritable eyes and dryness of the mouth. We mapped ALSG to a 22 cM region on chromosome 5p13.2-q13.1 in two multi-generation pedigrees to approach the molecular basis of the disorder. The candidate region coincided with the locus for *fibroblast growth factor 10 (FGF10)* previously shown to be involved in murine organogenesis including the development of lacrimal- and salivary glands[2-4]. We identified a 53 kb deletion including exon 2 and 3 of *FGF10* in affected individuals from one of the two families analyzed. In the second family, a nonsense mutation in exon 3 of *FGF10* segregated with ALSG. A reinvestigation of the $Fgf10^{+/-}$ mice revealed absence of lacrimal glands and hypoplasia of the salivary glands. This report presents for the first time the phenotypic effect of mutations in the human *FGF10* gene. We suggest that haploinsufficiency of FGF10, possibly during a critical stage of development, results in hypoplasia or aplasia of the lacrimal- and major salivary glands.

ALSG has a variable expressivity[5] and patients may show a combination of aplasia/hypoplasia of the lacrimal-, parotid-, submandibular- and sublingual glands together with absence of the lacrimal puncta. Reduced or absent tear production results in irritable eyes and recurrent infections, if the nasolacrimal ducts or lacrimal puncta are missing there might be epiphora (tear overflow). Xerostomia (dryness of the mouth) is a common symptom when the major salivary glands are affected which increases the risk of dental erosion and dental caries[6]. Other complications include periodontal disease,

infections of oral mucous membranes and disorders of smell, chewing and swallowing[5]. Individuals affected with ALSG are sometimes confused with or mis-diagnosed for the more prevalent disorder Sjogren syndrome, an autoimmune disorder characterized by keratoconjunctivitis sicca (KCS) and xerostomia[7]. Both sporadic and familial cases of ALSG have been described in the literature[5,6,8-12] but the incidence of the disorder is unknown. It has been suggested that the clinical variation of the disorder most likely reflects the pleiotropic effect of a single autosomal dominant gene[5]. The molecular genetic basis for ALSG has remained unclear and no previous molecular investigations of the disorder have been performed.

We recently identified one four-generation family (family 1) and one three-generation family (family 2) of Swedish origin with ALSG (**Fig. 1**). We describe here the clinical features of the disorder in these families, as well as the results from a genome-wide linkage scan and mutation analyses of *FGF10*. The phenotypes of the affected individuals are summarized in **Table 1**. In total, 16 individuals from both families were diagnosed with ALSG of which 14 were diagnosed after clinical examination (referred to below). Two family members (individual I:2 in family 1 and individual I:1 in family 2) were diagnosed with ALSG because of their medical history. Nine out of the 14 affected and clinically examined individuals fulfilled the criteria for KCS and eleven out of 14 displayed epiphora. In family 1 all affected members suffered from hyposalivation assessed by sialometry. The lacrimal- and major salivary glands were investigated by magnetic resonance imaging (MRI) (**Fig. 2**). The MRI displayed aplasia or hypoplasia of several major salivary glands in all affected individuals and 13/14 showed absent or

hypoplastic lacrimal glands. Hypoplasia/aplasia of the glands was accompanied by absence of one or several lacrimal puncta in 13/14 affected individuals. No additional abnormalities were observed and the affected individuals have a normal life span.

The ALSG inheritance in both families is autosomal dominant and the segregation pattern suggests a full penetrance. A genome wide screen with 400 polymorphic microsatellite markers, on DNA samples from family 1, revealed linkage for ALSG to a continuous pericentric region on chromosome 5, flanked by microsatellite markers D5S395 and D5S2046 (**Fig. 1**). We obtained a maximum cumulative lod score of 5.72 ($\theta = 0$) at the marker locus D5S398 for both families (**Supplementary Table 1**). The genetic distance between the markers D5S395 and D5S2046 is estimated to 22 cM corresponding to chromosome 5p13.2-q13.1. Within this region, the gene encoding fibroblast growth factor 10 (FGF10) is located[13]. Studies in mouse show that murine FGF10, with a 93% identity to human FGF10, is critical for the development of several organs including lacrimal- and salivary glands[2-4]. The *Fgf10* $^{-/-}$ mice die shortly after birth due to lack of lung development[4,14] and show absent lacrimal- and salivary glands, absent fore- and hind limbs, agenesis of pituitary and thyroid glands, dysgenic teeth, kidney, thymus, stomach, pancreas and inner ear as well as abnormal hair and skin[2,3]. Abnormal external genitalia development[15], anorectal malformations[16] and abnormal mammary gland formation[17] have also been reported. No abnormalities have previously been observed in *Fgf10*$^{+/-}$ mice.

We considered *FGF10* as a candidate gene for ALSG and sequenced the protein coding regions of the gene. Sequence analysis of the three exons of *FGF10* in samples from family 1 revealed no sequence alterations compared to sequences in the National Center for Biotechnology Information database. In order to identify deletions that might have escaped detection, we genotyped the family members for SNPs and microsatellite markers in the 5′and 3′ flanking regions of *FGF10* as well as in the introns. We found that the affected members of family 1 were hemizygous for microsatellite markers and SNPs flanking exon 2 and 3 (**Fig. 3a,b**). We amplified and sequenced the region containing the deletion-breakpoint and determined the size of the deletion to 53 kb, including exon 2 and 3, without the involvement of any flanking gene (**Supplementary Fig. 1** online). In family 2, DNA sequence analysis of the *FGF10* gene revealed a heterozygous stop mutation in exon 3 (R193X; 577C→T) resulting in a predicted truncated protein in the four affected members (**Fig. 3c**). This sequence alteration was excluded on 100 chromosomes from controls.

We then reexamined the *Fgf10*$^{+/-}$ mice previously described as apparently normal[3,14]. Adult female mice were dissected followed by a macroscopical and histological examination of the lacrimal and salivary gland apparatuses. The Fgf10$^{+/-}$ mice displayed aplasia of lacrimal glands together with atrophic or hypoplastic parotid, submandibular and sublingual glands (Fig. 4) Other internal organs including lung, liver, spleen, heart, stomach, thyroid, pancreas, intestines and ovaries were found normal in the *Fgf10*$^{+/-}$ mice.

In order to clarify if *FGF10* mutations are a cause of dry eyes/ dry mouth in sporadic cases with symptoms identical to those in our ALSG patients, we screened DNA samples from 74 individuals for mutations in the *FGF10* gene. These individuals had been evaluated and diagnosed with dry eyes and/or dry mouth, without fulfilling neither the preliminary European[18] nor the revised American-European criteria[19] for Sjogren syndrome. MRI of the lacrimal- and salivary glands for these patients was not performed. No sequence alterations in the coding region of the *FGF10* gene were found in samples from these individuals which suggest that *FGF10* mutations are uncommon in individuals with unspecific sicca syndromes.

In family 1 the affected patients are hemizygous for exon 2 and 3 of *FGF10* which supports haploinsufficiency as a mechanism behind ALSG. In family 2, the premature stop codon (R193X; 577C→T) in exon 3 predicts a truncated protein with a loss of 16 amino acids. The truncation abolishes one predicted cAMP- and cGMP-dependent protein kinase phosphorylation site (residues 194-197) and one predicted *N*-linked glycosylation site (residues 196-198), respectively[20]. Further more, the truncation eliminates a site for the high affinity interaction between FGF10 and fibroblast growth factor receptor 2b (FGFR2b) at residues 202 and 204[21,22]. If at all produced, the truncated FGF10 is most probably unstable or non-functional. Both FGF10 mutations identified in our families are consistent with haploinsufficiency as an underlying mechanism.

The results of the clinical examinations and the medical histories of the affected family members illustrate that one intact copy of *FGF10* is sufficient for development of the

extremities and other essential organs in humans. The restricted phenotype associated with heterozygosity for FGF10 in both humans and mice make us propose that the response to FGF10 is dosage-sensitive. This is most probably related to the specific embryonic stage and site of lacrimal- and salivary gland formation. We suggest that the levels of FGF10 derived from one allele are either sufficient for normal development and homeostasis of other organs dependent on FGF10, or that other factors can compensate for reduced levels of FGF10 in those organs. A possible explanation for the absence of generalized effects in heterozygotes is a functional overlap with other FGFR2b ligands such as FGF1, FGF3 and FGF7[23].

We observed a clinical variation from partial to complete absence of glands among carriers of the same *FGF10* mutation. This results in a spectrum of manifestations even within a single family and supports a previous report[5]. ALSG is a rare condition which may be difficult to diagnose in mild or sporadic cases. The main clinical features of ALSG are similar to the symptoms observed in Sjogren syndrome[7] but without an inflammatory component. In our families, several affected members were previously diagnosed with Sjogren syndrome. Identification of *FGF10* as the gene causing ALSG will hopefully result in increased diagnostic accuracy. In a larger context, this report clarifies the phenotypic effects of *FGF10* mutations which together with further identification of ALSG patients may lead to a better understanding of the mechanisms involved in lacrimal- and salivary gland formation.

Methods

Clinical studies. We obtained signed informed-consent forms, approved by the Ethical Committee at Uppsala University or by the collaborating Universities and Institutions, from all participating individuals.

Two families with ALSG were included in this study (**Fig. 1**). All members of family 1 underwent clinical examination except unaffected spouses and individual I:2, who was unable to co-operate and diagnosis was therefore ascertained through medical history. She had ectopic tear flow and received dentures at young age. In family 2, individuals II:3, III:1 and III:2 were examined clinically. Diagnoses of the other family members were ascertained through medical history. Individuals were considered as affected when they presented symptoms from both the lacrimal apparatus (lacrimal glands and/or lacrimal puncta) and the salivary glands. The oral examination included visual inspection of the puncta of the parotid-, submandibular- and sublingual ducts and sialometry to assess hyposalivation. The ophthalmologic examination included inspection of lacrimal puncta and ectopic tear flow as well as measurement of lacrimal fluid production with Schirmer's test and break up time (BUT). Possible epithelial lesions were studied with corneal staining and devitalized epithelial cells with Rose-Bengal test. Keratoconjunctivitis sicca (KCS) was diagnosed when an individual had pathological values on two or more of the three tests Schirmer's, BUT and Rose-Bengal. Lacrimal- and major salivary glands were visualized with magnetic resonance imaging (MRI).

We also analyzed samples from 74 unrelated individuals with symptoms of dry eyes/dry mouth. These patients had undergone labial salivary gland biopsy, autoantibody analysis and lacrimal- and salivary flow measurements. These patients fulfilled neither the preliminary European (Vitali et al. 1993) nor the revised American-European criteria (Vitali et al. 2002) for Sjogren syndrome.

Genome-wide linkage analysis and fine-mapping of the candidate region. Genomic DNA was isolated from peripheral blood lymphocytes according to standard procedures. A marker set containing 400 polymorphic microsatellite markers (Weber screening set version 6/Cooperative Human Linkage Center) at an average spacing of 10 cM were used for the genome-wide scan. PCR was performed according to the instructions of the manufacturer. Fine-mapping of the candidate region on chromosome 5 was performed with markers included in the deCODE genetic map[24]. We also developed new microsatellite markers from dinucleotide repeats surrounding the *FGF10* gene locus. Primer pairs were designed with the Primer3 program (primer sequences are available upon request). The primers were labeled with one of the fluorophores FAM, TET, HEX or NED. The PCR amplifications were carried out in a Peltier thermal cycler (PTC-225; MJ Research) or an ABI 877 integrated thermal cycler. We separated PCR products by electrophoresis on either an ABI 377 or an ABI 3700 DNA Analyzer (Applied Biosystems) and analyzed them with Genescan v3.1.3 and Genotyper v3.7 softwares (Applied Biosystems). The pedigrees were drawn with the Cyrillic software, version 2.1.3 (Cherwell Scientific Publishing Ltd, Oxford, UK) and the haplotype analysis was performed manually. Two-point LOD score calculations were performed with the

MLINK program of the LINKAGE package (version 5.1)[25] assuming an autosomal dominant inheritance, equal male to female recombination rate, full penetrance and a disease allele frequency of 0.00001. Equal allele frequencies of the genotyped markers were used in the calculations. We used the National Center for Biotechnology Information Entrez Genome Map Viewer and Ensembl Human Genome Server databases for localisation of microsatellite marker loci and for the identification of transcripts in the candidate region. The Genome Database was used for information on microsatellite markers and their primer sequences.

Mutation analysis of FGF10. Primer sequences and PCR conditions used for amplification of the exons of *FGF10*, SNPs in the *FGF10* region and the deletion-breakpoint in family 1 are available upon request. The sequence analysis of *FGF10* included the three exons and was performed on genomic DNA from individual I:2 of family 1 and individual II:3 of family 2 as well as DNA from 74 sporadic patients diagnosed with dry eyes/dry mouth. After the identification of the single nucleotide substitution (R193X; 577C→T) in exon 3 in individual II:3 of family 2, this exon was sequenced for the remaining family members and 50 controls. For sequencing, the PCR products were purified with the GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences). The sequencing was performed with an ABI 3700 DNA Analyzer (Applied Biosystems) using Big Dye Terminator v 3.1 Cycle Sequencing kit (Applied Biosystems). Base calling was done with the DNA Sequencing Analysis Software, version 3.7 (Applied Biosystems) and the sequences were aligned and analyzed with the Sequencher software, version 4.1 (Gene Codes Corporation). SNPs used for

deletion mapping of the *FGF10* region in family 1 were genotyped by sequencing or by endonuclease digestion. For endonuclease digestion, 20 µl of the PCR product was incubated with 5 U of the enzyme at the optimal cleavage temperature. The cleaved fragments were separated on a 2 % agarose gel and visualized by ethidium bromide staining. The deletion breakpoint was mapped and characterized using long-range PCR, with the Advantage 2 PCR enzyme system (Clontech) with primer sequences flanking the deletion, followed by sequencing.

Mutant mice. Three wildtype and two $Fgf10^{+/-}$ adult (8-9 weeks old) female mice were examined[3,14]. Dissection of different organs was performed for macroscopical examination. Hematoxylin and eosin staining of deparaffinized sections of the lacrimal- and salivary glands was performed according to standard procedures.

URLs. Primer 3 is available at http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi/. The National Center for Biotechnology Information Entrez Genome Map Viewer is available at http:///www.ncbi.nlm.nih.gov/mapview. The Ensembl Human Genome Server database is available at http://www.ensembl.org/. The Genome Database is available at http://www.gdb.org/.

GenBank accession numbers. Human FGF10, NM_004465, NP_004456.1; Human chromosome 5 clones containing FGF10, AC093537.2 and AC093289.2.

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References

1. Wiedemann, H.R. Salivary gland disorders and heredity. *Am J Med Genet* **68**, 222-4 (1997).
2. Makarenkova, H.P. et al. FGF10 is an inducer and Pax6 a competence factor for lacrimal gland development. *Development* **127**, 2563-72 (2000).
3. Ohuchi, H. et al. FGF10 acts as a major ligand for FGF receptor 2 IIIb in mouse multi-organ development. *Biochem Biophys Res Commun* **277**, 643-9 (2000).
4. Min, H. et al. Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to Drosophila branchless. *Genes Dev* **12**, 3156-61 (1998).
5. Ferreira, A.P. et al. Congenital absence of lacrimal puncta and salivary glands: report of a Brazilian family and review. *Am J Med Genet* **94**, 32-4 (2000).
6. Young, W. et al. Syndromes with salivary dysfunction predispose to tooth wear: Case reports of congenital dysfunction of major salivary glands, Prader-Willi, congenital rubella, and Sjogren's syndromes. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **92**, 38-48 (2001).
7. Bolstad, A.I. & Jonsson, R. Genetic aspects of Sjogren's syndrome. *Arthritis Res* **4**, 353-9 (2002).
8. Blackmar, F.B. Congenital atresia of all lacrimal puncta with absence of salivary glands. *Am J Ophthalmol* **8**, 139-140 (1925).
9. Higashino, H. et al. Congenital absence of lacrimal puncta and of all major salivary glands: case report and literature review. *Clin Pediatr (Phila)* **26**, 366-8 (1987).
10. Milunsky, J.M., Lee, V.W., Siegel, B.S. & Milunsky, A. Agenesis or hypoplasia of major salivary and lacrimal glands. *Am J Med Genet* **37**, 371-4 (1990).
11. Myers, M.A., Youngberg, R.A. & Bauman, J.M. Congenital absence of the major salivary glands and impaired lacrimal secretion in a child: case report. *J Am Dent Assoc* **125**, 210-2 (1994).
12. Caccamise, W.C. & Townes, P.L. Congenital absence of the lacrimal puncta associated with alacrima and aptyalism. *Am J Ophthalmol* **89**, 62-5 (1980).
13. Emoto, H. et al. Structure and expression of human fibroblast growth factor-10. *J Biol Chem* **272**, 23191-4 (1997).
14. Sekine, K. et al. Fgf10 is essential for limb and lung formation. *Nat Genet* **21**, 138-41 (1999).
15. Haraguchi, R. et al. Molecular analysis of external genitalia formation: the role of fibroblast growth factor (Fgf) genes during genital tubercle formation. *Development* **127**, 2471-9 (2000).
16. Fairbanks, T.J. et al. Fibroblast growth factor 10 (Fgf10) invalidation results in anorectal malformation in mice. *J Pediatr Surg* **39**, 360-5; discussion 360-5 (2004).
17. Mailleux, A.A. et al. Role of FGF10/FGFR2b signaling during mammary gland development in the mouse embryo. *Development* **129**, 53-60 (2002).
18. Vitali, C. et al. Preliminary criteria for the classification of Sjogren's syndrome. Results of a prospective concerted action supported by the European Community. *Arthritis Rheum* **36**, 340-7 (1993).

19. Vitali, C. et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* **61**, 554-8 (2002).
20. Bagai, S. et al. Fibroblast growth factor-10 is a mitogen for urothelial cells. *J Biol Chem* **277**, 23828-37 (2002).
21. Igarashi, M., Finch, P.W. & Aaronson, S.A. Characterization of recombinant human fibroblast growth factor (FGF)-10 reveals functional similarities with keratinocyte growth factor (FGF-7). *J Biol Chem* **273**, 13230-5 (1998).
22. Yeh, B.K. et al. Structural basis by which alternative splicing confers specificity in fibroblast growth factor receptors. *Proc Natl Acad Sci U S A* **100**, 2266-71 (2003).
23. De Moerlooze, L. et al. An important role for the IIIb isoform of fibroblast growth factor receptor 2 (FGFR2) in mesenchymal-epithelial signalling during mouse organogenesis. *Development* **127**, 483-92 (2000).
24. Kong, A. et al. A high-resolution recombination map of the human genome. *Nat Genet* **31**, 241-7 (2002).
25. Lathrop, G.M. & Lalouel, J.M. Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* **36**, 460-5 (1984).

Tables

Table 1 Summary of clinical examination of affected individuals in family 1 and 2.

										Result of MRI analysis							
	KCS		Epiphora		Hypo-salivation	Lacrimal puncta				Lacrimal gland		Parotid gland		Submandi-bular gland		Sublingual gland	
	R	L	R	L		R_S	R_i	L_S	L_i	R	L	R	L	R	L	R	L
Family 1																	
II:1	+	+	+	+	+	+	+	+	+	-	-	hyp	hyp	-	-	+	+
II:3	+	+	-	-	+	-	+	-	+	+	+	hyp	hyp	-	-	+	+
II:5	-	-	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+
II:7	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	+	+
III:2	+	-	+	+	+	+	-	-	-	-	-	-	hyp	-	+	+	+
III:3	+	+	+	+	+	-	-	-	-	-	-	-	-	hyp	-	+	+
III:7	+	+	-	-	+	+	-	+	-	-	-	-	-	+	+	+	+
III:10	+	+	-	-	+	-	+	+	+	-	-	-	-	hyp	-	+	+
IV:1	-	+	+	-	+	-	-	-	+	-	-	hyp	-	-	-	+	+
IV:2	-	-	+	+	+	-	-	-	+	-	-	-	-	+	+	-	-
IV:3	-	-	-	+	+	+	+	+	-	-	hyp	-	-	-	-	-	-
Family 2																	
II:3	+	-	+	+	ND	-	-	-	-	-	hyp	-	-	-	-	+	+
III:1	-	-	+	+	ND	-	-	-	-	-	-	-	-	-	-	+	+
III:2	-	-	+	+	ND	-	-	-	-	-	hyp	-	-	-	-	+	+

R, right; L, left; $_S$, superior; $_I$, inferior; +, present; -, absent; hyp, hypoplastic; ND, not determined

Supplementary Table 1 Two-point lod scores assuming full penetrance and autosomal dominant inheritance between chromosome 5 markers and the locus for aplasia of lacrimal- and salivary glands. These results are cumulative for the two families.

	Lod score at θ =				
Marker	0.00	0.10	0.20	0.30	0.40
D5S395	-∞	2.27	1.87	1.27	0.60
D5S1998	2.98	2.22	1.41	0.64	0.12
D5S2494	4.82	4.04	3.17	2.20	1.12
D5S2063	0.60	0.46	0.32	0.17	0.04
D5S2106	0.51	0.51	0.40	0.29	0.16
D5S2076	4.52	3.69	2.78	1.78	0.74
D5S2102	4.82	3.99	3.08	2.08	1.01
D5S398	5.72	4.76	3.69	2.51	1.25
D5S2500	3.91	3.18	2.37	1.48	0.58
D5S2046	-∞	2.23	1.77	1.13	0.47

Figure legends

Figure 1 ALSG family pedigrees and segregation of chromosome 5 marker loci. Filled symbols denote affected individuals; open symbols denote unaffected individuals. The most likely haplotypes and recombination events are illustrated below the symbols. The disease haplotypes associated with ALSG are indicated by black bars.

Figure 2 Magnetic resonance imaging of lacrimal- and salivary glands. (**a,b**) Magnetic resonance imaging T1-weighted sequence demonstrating (**a**) presence of lacrimal glands (arrows) in a control and (**b**) absence of lacrimal glands in individual III:2 in family 2. (**b,c**) Magnetic resonance imaging T2-weighted sequence demonstrating (**b**) presence of parotid glands (arrows) in a control and (**c**) absence of parotid glands in individual IV:3 in family 1.

Figure 3 Identification of *FGF10* mutations in family 1 and 2. **(a)** Schematic overview of the *FGF10* gene and the 53 kb deletion inherited with ALSG in family 1 (figure not drawn to scale). Black boxes denote exons 1-3 and deletion breakpoints are indicated by wavy vertical lines. The deletion was characterized by genotyping of dinucleotide repeats and SNPs, long-range PCR and sequencing. The two dinucleotide repeats (TA53 and CA17) and the three SNPs (rs10060548, rs6881797 and rs2290070) showed loss of heterozygosity in several affected family members. Long-range PCR with a forward primer (F) situated on one side of the deletion breakpoint and reverse primers (R1, R2, R3 or R4) situated 3' of the *FGF10* were used to generate amplicons across the

breakpoint. The PCR product obtained with primers F and R2 spanned the breakpoint and sequencing defined the breakpoint position to within 6 nucleotides (boxed). The exact position of the breakpoint could not be determined due to presence of identical sequences flanking the deletion. (**b**) Genotyping of the SNP rs6881797, located within the deletion of family 1 and 37 bp 3′ of exon 2, by digestion with *Bsr*I. Undigested PCR product (376 bp) corresponds to the T allele and digested PCR product (329 bp) corresponds to the A allele. The absence of a paternal A allele in individual III:7 in family 1 indicates hemizygosity of rs6881797. (**c**) The upper sequence chromatogram illustrates the heterozygous (R193X; 577C→T) mutation found in the affected members of family 2. The lower sequence chromatogram illustrates the same sequence from an unaffected individual.

Figure 4 Salivary and lacrimal gland apparatuses of wildtype and *Fgf10*$^{+/-}$ mice. (**a**) Macroscopic observation of wild-type (left) and *Fgf10*$^{+/-}$ (right) adult mice. Ventral view of the mandibular region. 1, lacrimal; 2, parotid; 3, sublingual; 4, submandibular glands. The asterisk denotes the expected site for the lacrimal gland absent in the heterozygote. Other glands are hypoplastic in the heterozygote. (**b**,**c**) Dissected salivary and lacrimal glands from the wild-type (**b**) and *Fgf10*$^{+/-}$ (**c**) mice numbered as in (**a**). The salivary glands from the *Fgf10*$^{+/-}$ are hypoplastic and the lacrimal gland is absent. (**d-i**) Histology of wild-type (**d**,**f**,**h**) and *Fgf10*$^{+/-}$ (**e**,**g**,**i**) glands. (**d**) Wild-type lacrimal (lg) and parotid (pg) glands. (**e**) The lacrimal gland is replaced by adipose tissue (asterisk) in the *Fgf10*$^{+/-}$ mouse. (**f**) Wild-type parotid gland. (**g**) *Fgf10*-heterozygote parotid gland which appear atrophic. (**h**,**i**) Submandibular glands exhibit similar morphology in the wild-type and

heterozygote, as is the case with sublingual glands (not shown). Scale bars, 0.5mm (**d,e**); 0.1mm (**f-i**).

Supplementary Figure 1 Amplicons generated by long-range PCR of the FGF10 region in individual III:2 of family 1 and control. The same forward primer, situated 5'of the deletion breakpoint, was used in each PCR reaction together with different reverse primers (R1, R2, R3 or R4). The forward and reverse primers are situated 52-54 kb apart according to the National Center for Biotechnology Information database.