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Recurrent Acute Otitis Media

Epidemiological and Clinical Alterations with Some Genetical and Bacteriological Aspects

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ACADEMIC DISSERTATION

To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the main auditorium of Building K,
Medical School of the University of Tampere,
Teiskontie 35, Tampere, on January 10th, 2003, at 12 o'clock.

Acta Universitatis Tamperensis 901
University of Tampere
Tampere 2003

ACADEMIC DISSERTATION
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http://granum.uta.fi

Cover design by
Juha Siro

Printed dissertation
Acta Universitatis Tamperensis 901
ISBN 951-44-5526-6
ISSN 1455-1616

Electronic dissertation
Acta Electronica Universitatis Tamperensis 221
ISBN 951-44-5527-4
ISSN 1456-954X
http://acta.uta.fi

Tampereen yliopistopaino Oy Juvenes Print
Tampere 2003

To the joy of children

Abstract

Background: Acute otitis media (AOM) is one of the most common childhood illnesses. Nearly all children experience at least one otitis episode, but only every tenth child suffers from recurrent acute otitis media (RAOM). Recently, it has become evident that RAOM has a genetic background. Several external factors also predispose a child to middle-ear infections. Changes in these risk factors may lead to an alteration in the incidence of AOM in the population. During the last few decades, the occurrence of AOM seems to have been rising. This has, however, been difficult to prove because of a lack of surveys in the same population over time.

Subjects and methods: The alteration in the occurrence of AOM was studied by registering retrospectively all the attacks of AOM among children under ten years of age in the same geographical area (with the total population of about 21 000) during two 12-month periods – from June 1, 1978, to May 31, 1979, and from June 1, 1994, to May 31, 1995. In addition, the symptoms, the clinical picture, and the given treatments were analysed. Polymorphism of tumor necrosis factor α, interleukin-1α, interleukin-1β and interleukin-1 receptor antagonist genes were studied in 63 subjects with a history of recurrent episodes of otitis media. The controls were 400 healthy blood donors. Nasopharyngeal samples for bacterial culture were taken from 306 children suffering from RAOM, to evaluate the occurrence of antibiotic resistance of otitis pathogens.

Results: The occurrence of AOM rose by 68% (95% CI 53-79%) between the study periods 1978-79 and 1994-95. In 1978-79, 2% of the children with AOM during the study year experienced at least three recurrences, the corresponding figure being 6% in 1994-95 (OR = 3.7, 95% CI = 1.6-8.5). The proportion of otitis patients with acute otorrhea and/or fever was lower in 1994-95. Prescribing penicillin-V as a primary treatment decreased from 80% (1978-79) to 11% (1994-95) in favour of broad-spectrum antibiotics.

A clear association between any studied cytokine genes and RAOM was not found, except in a subgroup of non-allergic individuals in whom interleukin-1α (-889) gene allele frequencies differed from those in the control group (p=0.03). This was mainly due to an increasing occurrence of common 1.1 genotype (60.9% vs. 41.8%, OR 2.2, 95% CI 1.3-4.1).

Nasopharyngeal bacterial culture and susceptibility testing revealed that of the isolated *Moraxella catarrhalis* and *Haemophilus influenzae* strains 93% and 43% produced β-lactamase, respectively. Of the *Streptococcus pneumoniae* strains, 25% were non-susceptible (I/R) to penicillin. Concomitant nasopharyngeal carriage of β-lactamase producing *Moraxella catarrhalis* seemed to prevent the emergence of penicillin resistance among *Streptococcus pneumoniae* strains.

Conclusion: In the past two decades, the occurrence - and especially the recurrences - of AOM have increased strongly, but its clinical picture has become milder. Still, children are treated more aggressively. An affluent use of broad-spectrum antibiotics in the treatment of AOM may lead to alarmingly high bacterial resistance figures. Interleukin-1α gene polymorphism may be associated with a liability to contract recurrent otitis episodes in a non-allergic subgroup of patients.

Lyhennelmä

Tausta: Äkillinen välikorvantulehdus on yksi yleisimmistä lapsuusajan sairauksista. Lähes jokainen lapsi sairastaa yhden välikorvantulehduksen, mutta vain joka kymmenes lapsi sairastuu toistuviin äkillisiin välikorvantulehduksiin. Viimeaikaiset tutkimukset ovat osoittaneet, että geneettiset tekijät vaikuttavat alttiuteen sairastua korvatulehdukseen. Myös monet ulkoiset tekijät lisäävät sairastumisriskiä, ja muutokset näissä riskitekijöissä voivat johtaa vaihteluun korvatulehdusten esiintymisessä väestössä. Viime vuosikymmeninä äkillisen välikorvantulehduksen esiintyvyys vaikuttaisi lisääntyneen. Luotettavaa tutkimusnäyttöä tästä ei kuitenkaan ole.

Menetelmät: Korvatulehduksen esiintymisen muutosta tutkittiin rekisteröimällä Kurikassa ja Jalasjärvellä (tutkimusalueen väkiluku n. 21000) kaikki sairastetut välikorvantulehdukset, niiden oireet ja annettu hoito 12 kuukauden jaksoilta vuosilta 1978-79 ja 1994-95. Korvatulehduksen genetiikkaa selvitettiin vertaamalla 63:n toistuvaa välikorvantulehdusta sairastavan henkilön sytokiinigeenien (tuumorinekroositekijä α, interleukiini-1α ja -β sekä interleukiini-1 reseptoriantagonisti) polymorfismia 400:n terveen verenluovuttajan vastaavien geenien polymorfismiin. Korvatulehdusta aiheuttavien bakteerien antibioottiresistenssiä tutkittiin ottamalla nenänielun bakteeriviljelynäyte 306:lta toistuvan välikorvantulehduksen vuoksi kitarisa/putkitusleikkaukseen tulevalta lapselta.

Tulokset: Äkillisen välikorvantulehduksen esiintyvyys vuosien 1978-79 ja 1994-95 välillä lisääntyi 68% (95%:n luottamusväli 53-79%), ja erityisesti lisääntyivät toistuvat välikorvantulehdukset: esimerkiksi kolme uusintatulehdusta sairastaneiden lasten määrä kolminkertaistui tutkimusjaksojen välillä (OR 3,7, 95%:n luottamusväli 1,6-8,5). Kuume sekä äkillinen korvan märkävuoto korvatulehduksen yhteydessä oli harvinaisempaa jälkimmäisellä tarkastelujaksolla. V-penisilliinin käyttö korvatulehduksen ensivaiheen lääkkeenä väheni 80%:sta 11%:iin. Vastaavasti laajakirjoisten antibioottien käyttö lisääntyi.

Selvää yhteyttä tutkittujen sytokiinigeenien ja korvatulehdusalttiuden välillä ei todettu paitsi ei-allergisilla toistuvista korvatulehduksista kärsivillä henkilöillä, joilla interleukiini-1α geenin alleelifrekvenssi erosi tilastollisesti merkitsevästi kontrolliryhmän vastaavan geenin alleelifrekvenssistä (p=0,03). Tämä johtui erityisesti yleisen 1.1 genotyypin suuremmasta esiintyvyydestä korvatulehdusryhmässä (60,9% vs. 41,8%, OR 2,2, 95%:n luottamusväli 1,3-4,1).

Nenänielun bakteeriviljelyn herkkyysmäärityksen perusteella 93% eristetyistä *Moraxella catarrhalis* kannoista ja 43% *Haemofilus influenzae* kannoista tuotti beetalaktamaasia. Lisäksi pneumokokkikannoista neljäsosassa todettiin alentunutta herkkyyttä (I/R) penisilliinille. Beetalaktamaasia tuottavan *Moraxella catarrhaliksen* nenänielukantajuus näytti estävän pneumokokin penisilliiniresistenssin kehittymistä.

Yhteenveto: Viimeisten kahden vuosikymmenen aikana korvatulehdusten esiintyvyys on lisääntynyt selvästi, ja erityisesti toistuvat välikorvantulehdukset ovat yleistyneet. Vaikka korvatulehdukset ovat nykyään lievempiä kuin aikaisemmin, lapsia hoidetaan yhä laajakirjoisemmilla antibiooteilla, mikä johtaa bakteeriresistenssin hälyttävään lisääntymiseen. Interleukiini-1α geenin polymorfismi saattaa olla yhteydessä ei-allergisten henkilöiden alttiuteen sairastua toistuviin välikorvantulehduksiin.

Contents

Abbreviations

AOM	acute otitis media
CI	confidence interval
COM	chronic otitis media
COME	chronic otitis media with effusion
ET	eustachian tube
GAS	group A streptococcus
HLA	human leukocyte antigen
IL	interleukin
IgG	immunoglobulin G
MEE	middle-ear effusion
OM	otitis media
RAOM	recurrent acute otitis media
RNA	ribonucleic acid
SOM	secretory otitis media
TNF	tumor necrosis factor

List of original publications I-IV

This thesis is based on the following publications, referred to in the text by their Roman numerals, and on some additional data.

I Joki-Erkkilä VP, Laippala P, Pukander J: Increase in paediatric acute otitis media diagnosed by primary care in two Finnish municipalities-1994-5 versus 1978-9. *Epidemiol Infect* 1998; 121: 529-534

II Joki-Erkkilä VP, Pukander J, Laippala P: Alteration of clinical picture and treatment of pediatric acute otitis media over the past two decades. *Int J Pediatr Otorhinolaryngol* 2000; 55: 197-201

III Joki-Erkkilä VP, Puhakka H, Hurme M: Cytokine gene polymorphism in recurrent acute otitis media. *Arch Otolaryngol Head Neck Surg* 2002; 128: 17-20

IV Joki-Erkkilä VP, Aittoniemi J, Vuento R, Puhakka H: ß-lactamase producing *Moraxella catarrhalis* may prevent the emergence of penicillin-resistant *Streptococcus pneumoniae* in children with recurrent acute otitis media. *Int J Pediatr Otorhinolaryngol* 2002; 63: 219-222

Introduction

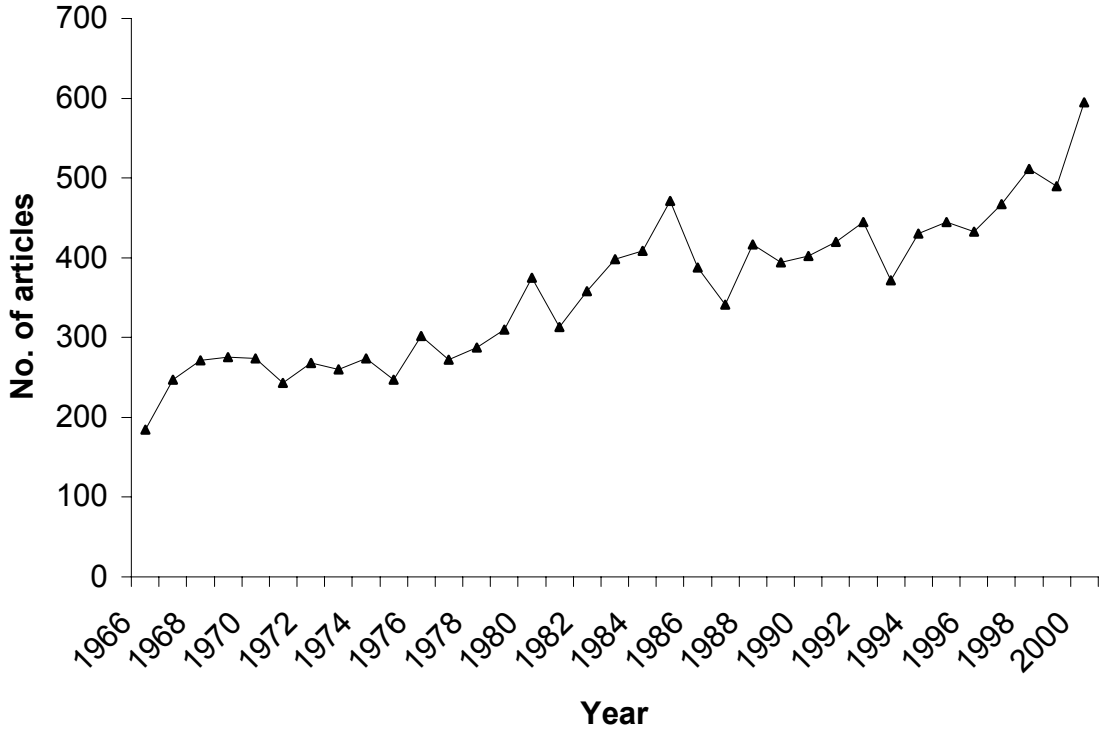
Otitis media (OM) is the most common reason for children to receive antibiotics or undergo surgical care. Still, after many years of intensive research work (Figure 1), it is not clear why some children contract recurrent episodes of acute otitis media (AOM). Not until recently has it become evident that recurrent acute otitis media (RAOM) has a genetic background. It has, however, been difficult to discover specific genetic markers associated with the development of this disorder. It seems reasonable that there are many genes that contribute to the overall phenotype. The candidate genes are likely to be those which control mucosal immunity.

In addition to a genetic background, several external factors have been implicated as risk factors for AOM. Changes in risk factors may lead to incidence differences of AOM in the population. In recent decades, several epidemiological studies indicate that the occurrence of OM is increasing. Nevertheless, this has been difficult to prove because of a paucity of surveys in the same population over time. Any comparison between studies made in different years and different places is complicated and biased because of several factors, including differences in study designs, demography and diagnostic criteria.

In this thesis, epidemiological alterations of AOM were evaluated. The changes in the occurrence and the clinical picture of AOM were estimated by registering retrospectively all AOM episodes in the same geographical area in two 12-month periods 15 years apart. The polymorphism of different cytokine genes in RAOM was studied. In addition, the occurrence of resistance of otitis pathogens was screened in children with RAOM.

The accurate knowledge of epidemiological alterations of AOM highlights the magnitude of this problem and brings up the concrete importance of different risk factors of AOM. Knowledge of RAOM susceptibility genes could help to identify the children with a high risk for OM, provide new insights into the pathogenesis of the disease, and lead to more focused treatments of OM.

Figure 1. Number of scientific articles about otitis media per publication year (Medline search from 1966 to 2000 with a key word “otitis media”)

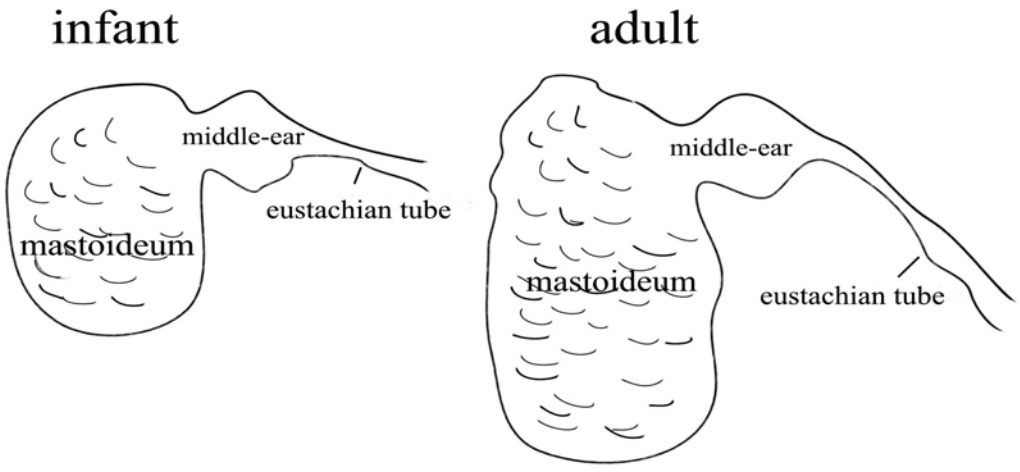


Review of the literature

1. Terminology and classification of otitis media

Otitis media (OM) is a general term used to describe any inflammatory process involving the middle-ear cleft (Figure 2). The main categories of OM are 1) acute otitis media (AOM), 2) secretory otitis media (SOM), which is also named "chronic otitis media with effusion" (COME), and 3) chronic otitis media (COM) with or without cholesteatoma. However, in medical literature, several terms are used to refer to the various stages of OM. The International Classification of Diseases (ICD, Table 1) is an attempt to produce a universal classification schema of otitis media, but it fails to provide a logically organized set of terms to describe the inflammatory process of the middle-ear in an exact way, and it is not well-accepted among otolaryngologists (Harkness and Topham 1998).

Figure 2. The middle-ear cleft is a collective term for a continuous space including the eustachian tube, the middle-ear (tympanum), and mastoid air cells



The following definitions are commonly used for OM: AOM is a condition with acute middle ear effusion (MEE) and acute symptoms and signs of infection e.g., fever, pain, restless sleep, irritability, tugging or rubbing of ears. AOM may also be accompanied with acute discharge from the ear. Recurrent acute otitis media (RAOM) is defined as a condition with at least 3 AOM episodes in 6 months or 4 episodes within a year (Klein 1984, Alho 1997). SOM (or COME) means that MEE has lasted at least 2-3 months behind intact tympanic membrane. A permanent perforation of tympanic membrane with recurrent or constant purulent discharge indicates COM. This can be associated with cholesteatoma, a condition where keratinising stratified squamous epithelium accumulates in the middle-ear cleft.

In practice, the classification between AOM, RAOM and SOM can sometimes be difficult, since, after a single acute episode, effusion in the middle-ear cavity may be observed after several weeks in some children. Secondly, in many cases SOM is a consequence of frequent acute middle-ear infections. Thirdly, SOM is often accompanied with recurrent acute signs and symptoms of an infection.

The current classification of OM has been criticized, because this has led to the investigation of OM by researches as a fairly homogenous entity, although fluid in the middle-ear is only the result of different pathologic phase (Pellman 1999). It is obvious that an infant with a bilateral AOM has an entirely different kind of prognosis than a 12-year-old child with an unilateral acute disease. Furthermore, mucoid effusion in tympanum in a 14-month-old child with a history of RAOM is a different disease than mucoid effusion in a 7-year-old school-child without a history of AOM attacks.

Some attempts have been made to a more detailed grading of AOM. Kaleida et al. (1991) divided otitis episodes into severe and non-severe in a clinical trial of the efficacy of antibiotics and myringotomy in the treatment of AOM. The grading was based on otalgia scoring points and the child's temperature (≥39ºC orally or ≥39.5ºC rectally, was classified as severe). A grading based on the subject's body temperature and the severity of the otalgia was also suggested by Bluestone and Klein (2001). Pellman (1999) introduced a more precise classification system, which includes 1) the presence or absence of a bilateral disease, 2) the age of the child, 3) the season, 4) the presence or absence of fever.

Health care professionals must be able to communicate with each other both clinically and scientifically. A universally accepted classification and staging system is a cornerstone to achieve accurate evidence-based information about OM.

Table 1. The ICD-10 classification of otitis media

H65 Nonsuppurativa otitis media
H65.0 Acute serous otitis media *(otitis media secretoria [sub]acuta)*
H65.1 Other acute nonsuppurative otitis media *(otitis media non purulenta [sub]acuta NAS)*
H65.2 Chronic serous otitis media *(otitis media serosa chronica)*
H65.3 Chronic mucoid otitis media *(otitis media mucoides chronica, glue ear, otitis media secretoria chronica)*
H65.4 Other chronic nonsuppurativa otitis media *(otitis media non purulenta chronica NAS)*
H65.9 Nonsuppurativa otitis media, unspecified *(otitis media exsudativa, otitis media catarrhalis, otitis media mucosa, otitis media secretoria, otitis media seromucosa, otitis media serosa, otitis media transsudativa, otitis media non purulenta)*
H66 Suppurativa otitis media
H66.0 Acute suppurativa otitis media *(otitis media suppurativa acuta)*
H66.1 Chronic tubotympanic suppurativa otitis media *(otosalpingitis chronica suppurativa)*
H66.2 Chronic atticoantral otitis media *(otitis media atticoantralis suppurativa chronica)*
H66.3 Other chronic suppurativa otitis media *(alia otitis media purulenta chronica, otitis media suppurativa chronica NAS)*
H66.4 Suppurativa otitis media, unspecified *(otitis media suppurativa NAS)*
H66.9 Otitis media, unspecified *(otitis media non specificata, otitis media NAS, otitis media acuta NAS, otitis media chronica NAS, otitis media NAS)*
H71 Middle ear cholesteatoma *(cholesteatoma auris media)*
H72 Tympanic membrane perforation *(perforatio membrana tympanicae)*
H73 Other disorders of tympanic membrane
H73.0 Acute myringitis *(myringitis acuta)*

2. *Pathogenetic aspects of acute and recurrent otitis media*

General considerations

The understanding of the pathogenesis of AOM, is the basis of discovering effective strategies in the management of this disease. To elucidate the pathogenesis of AOM, the following commonly accepted points must be respected: 1) Normally the middle-ear cavity is sterile, and this sterility is maintained by the mucociliary system and by the enzymes and antibodies secreted by the epithelial cells of the eustachian tube (ET) and the middle-ear (Lim et al. 2000). 2) AOM is usually a bacterial infection (Table 2). 3) It is often preceded by a respiratory virus infection (Henderson et al. 1982, Heikkinen et al. 1995). 4) The same otitis pathogens that are found in MEE are also found in the nasopharynx (Loos et al. 1989). 5) Although AOM can occur at any age, it is at its most common in young children, particularly at the age of 6-24 months (Pukander et al. 1982, Lundgren and Ingvarsson 1983, Teele et al. 1989). 6) A genetic component is involved in the predisposition to middle-ear infections (Casselbrant and Mandel 2001).

AOM is considered to be a bacterial disorder since a normal healthy middle-ear cavity is sterile, but during otitis episodes pathogenic bacteria can be usually cultured in MEE (Luotonen et al. 1981, Bluestone and Klein 2001, Kilpi et al. 2001). *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella* (formerly *Neisseria* and *Branhamella*) *catarrhalis* are the most common isolates world-wide (Table 2). Furthermore, MEE, which are negative by standard culture methods, can contain metabolically active bacteria detected by the presence of the bacterial messenger RNA (Rayner et al. 1998).

Clinical experience as well as epidemiological studies suggest that otitis episodes are most often preceded by a viral respiratory infection. Especially respiratory syncytial, influenza A and B, and adenovirus infections confer an increased risk for AOM in the subsequent 2 weeks (Henderson et al. 1982, Heikkinen et al. 1995).

However, viruses as a sole pathogen have been recovered from MEE only in 6% of the cases, but more often with pathogenic bacteria (Heikkinen 2000). Animal studies have been helpful in clarifying the role of viruses in AOM. In studies with chinchillas the intranasal inoculation of *S. pneumoniae* together with influenza A virus resulted in OM in 67% of cases, whereas the inoculation of *S. pneumoniae* alone resulted in the development of OM in 21% of cases, and only 4% of chinchillas inoculated with influenza A virus alone developed OM (Giebink et al. 1980). The predisposing effect of respiratory virus infection to OM is probably due to the ability of viral infections to destroy cilia cells (Giebink et al. 1987). In addition, virus infections can increase the adherence of pathogenic bacteria by triggering inflammatory activation of the epithelium. Consequently, this releases inflammatory cytokines, which have been shown to increase the adherence of bacteria to epithelial cells (Cundell et al. 1995, Tong et al. 1999).

Table 2. Bacterial isolates from middle-ear effusions in children with AOM Modified from Bluestone and Klein (2001) and Kilpi et al. (2001)

Bacterial pathogen	% of isolations
Streptococcus pneumoniae	26-38
Haemophilus influenzae	23-27
Moraxella catarrhalis	10-23
Group A streptococcus	≤3
Staphylococcus aureus	2-5
No growth or nonpathogenes	28
At least two pathogens simultaneously	8

From the pathogenic point of view, it is important to give an explanation how the bacteria enter the middle-ear. Because the nasopharyngeal carriership of pathogenic bacteria is a risk factor for AOM (Harabuchi et al. 1994, Faden et al. 1997), and as it has been shown with genetic fingerprints that the same bacteria that has been identified

in the middle-ear can be found in the nasopharynx (Loos et al. 1989), it is logical to assume that bacteria enter the middle-ear from the nasopharynx via the ET.

Eustachian tube

The three main functions of the ET are 1) pressure equalization, 2) the transportation of fluid from the middle-ear to the nasopharynx using mucociliary activity and 3) the prevention of reflux from the nasopharynx to the middle-ear (Bluestone 1996). The dysfunction of the ET is probably one of the most significant pathogenetic factors in the development of OM. Because OM is mostly a paediatric disease, it has been logical to compare a child's ET to that of an adult. Bluestone et al. (1974) showed that young children have shorter, straighter and more compliant ET's than adults (Figure 2). This permits a reflux from the nasopharynx to the middle-ear with the consequence of bacterial contamination. By comparison it is a well-known fact that urinary track infections are more common among females than among males with longer urethra (Hooton 2000).

On the other hand, it has been shown that children with recurrent otitis episodes have less effective active tubal function (= muscular opening function) than the controls (Stenström et al. 1991). This has been called ET dysfunction, but it should be more appropriately considered a sequel of an ET inflammation (Bernstein 1999).

An infection in the nasopharynx and the ET results in a congestion of mucosa leading to the obstruction of the ET. Because the middle-ear is a non-collapsible "gas pocket" surrounded by mucous membrane which absorb gases into microcirculation, obstruction of the ET causes negative middle-ear pressure (Bluestone 1996). Since the drainage function of the ET is also impaired, secretions in the middle-ear tend to accumulate (Bluestone 1996, Bernstein 1999). In addition, negative middle-ear pressure can lead to an aspiration of the nasopharyngeal secretions, including pathogenic bacteria.

Nasopharyngeal bacterial flora

The human nasopharynx is the main reservoir of otitis pathogens. In newborn babies the nasopharynx is sterile, but a microbial colonization begins immediately after birth. The initial colonization depends on such factors as the route of delivery, the type of nourishment received and the degree of exposure to the hospital environment (Mackowiak 1982).

Normal nasopharyngeal bacterial flora refers to the population of microbes, such as α haemolytic streptococci, diphteroides and *Neisseria spp*, inhabiting on mucosal surfaces of a healthy individual (Mackowiak 1982). It is noteworthy that potentially pathogenic bacteria like*, S. pneumoniae, H. influenzae* and *M. catarrhalis* are also considered to be part of the normal flora. Normal viral flora is generally not believed to exist in human beings.

Nasopharyngeal colonization with otitis pathogens is an important risk factor for OM. Faden et al. (1997) examined the colonization of infants by *S. pneumoniae, H. influenzae* and *M. catarrhalis* and found that those colonized before three months of age were at an increased risk of OM compared with children colonized with the pathogen later.

Normal bacterial flora provides a natural defence mechanism against infection. Low nasopharyngeal levels of α haemolytic streptococci have been associated with an increased susceptibility to OM (Bernstein et al. 1993a). The balance of normal flora is altered during viral infections. During the episode of otitis media, the carriage of otitis pathogens increases, and, at the same time, the carriage of non-pathogens of the flora decline (Faden et al. 1990). Recently, a Finnish study group followed 329 children from ages 2 to 24 months taking repeated nasopharyngeal cultures to evaluate the natural course of the carriage of *S. pneumoniae* (Syrjänen et al. 2001). During the follow-up period, the carriage of this pathogen fluctuated from 9-100%. It 1) increased with age, 2) was lower during health than during a respiratory infection and 3) was at its highest during AOM, especially when AOM was caused by *S. pneumoniae.*

Adenoids

Adenoids are part of Waldeyer's ring, a term for lymphoid tissue including adenoids (pharyngeal tonsils), palatine tonsils and the lingual tonsil (Wiatrak and Woolley 1998). Adenoids are not usually apparent in early infancy, but they gradually undergo hypertrophy and hyperplasia to reach their relatively greatest size by the age of 5 years. After this adenoids tend to regress along with increasing age of a child.

For years, it was assumed that adenoid tissue contributes to OM mainly by obstructing the ET. Especially a correlation between OME, requiring the insertion of ventilation tubes, and laterally hypertrophic adenoids abutting the torus tubarius has been suggested (Wright et al. 1998). However, many authors have failed to demonstrate any association between the actual size of adenoids and OM (Roydhouse 1980, Gates et al. 1989, Mandel et al. 1992, Gates et al. 1992).

In recent literature, the concept that adenoid tissue probably acts as a source of infection has become more popular (Gates et al. 1992, Linder et al. 1997, Brook et al. 2000). Brook et al. (2000) studied qualitative and quantitative microbiology of adenoid tissue and found that the number of potential pathogens was higher in adenoids of children with RAOM, recurrent adenotonsillitis and obstructive adenoid hypertrophy than in the controls. Most episodes of AOM follow an upper respiratory track infection-induced bacterial colonization of the nasopharynx with a secondary colonization of the middle-ear. The rationale for the removal of the adenoids in the treatment of OM is to eliminate the infection focus.

Adenoids are an affluent source of inflammatory mediators like immunoglobulins and cytokines (Bernstein et al. 1998). It has been shown that children with RAOM may have a decreased nasopharyngeal immunoglobulin (Harabuchi et al. 1994) and pro-inflammatory cytokine (Lindberg et al. 1994) production compared to the healthy controls, but it is not clear if this is secondary to an infectious process. The immunologic role of adenoids in the pathogenesis of AOM is for the moment poorly understood.

Cytokines

Cytokines are bioactive proteins widely mediating host responses to inflammatory stimuli. In the pathogenesis of an inflammation in the respiratory track they regulate proliferation, chemotaxis and the activation of inflammatory cells (Nicod 1993). There are three main pro-inflammatory cytokines; the tumor necrosis factor (TNF) α, the interleukin (IL) -1 and IL-6 and anti-inflammatory cytokines like interleukin-1 receptor antagonist (IL-1Ra) and IL-10 which downregulate the inflammatory process (Hurme et al. 1998). IL-1 appears in two forms, IL-1α and IL-1β. These proteins have a similar profile of functions, but IL-1α is primarily cell associated while IL-1β is the primary secreted form of IL-1 (Dinarello 1998).

There is ample evidence that cytokines are key mediators in the pathogenesis of an OM-related inflammation. In the last few years, a number of studies have demonstrated significant amounts of several cytokines, like IL-1β, IL-2, TNF-α, interferon (IF)-γ, IL-6, and IL-8 in middle ear effusions (Yellon et al. 1991, Juhn et al. 1992, Yellon et al. 1992, Juhn et al. 1994, Maxwell et al. 1994, Yellon et al. 1995). Various types of cytokines, like IL-1β, IL-6, and TNF-α have also been detected from nasopharyngeal secretions of RAOM children (Lindberg et al. 1994). High concentrations of TNF-α in the MEE seems to correlate with the persistence of SOM, because children undergoing repeated tympanostomy-tube placements had nearly 14 times higher average levels of TNF-α than children undergoing their first tympanostomy (Yellon et al. 1991).

While TNF-α plays an important role in prolonged and chronic stages of OM, IL-1 seems to be a key mediator in the acute phase of infection. In the chinchilla model of OM, IL-1β is the earliest pro-inflammatory cytokine detected in MEF (Sato et al. 1999). In AOM concentrations of IL-1 are 3 times higher in culture-positive MEF, compared culture-negative ones and the concentration of IL-1 decreases along with eradication of bacterial pathogens with antimicrobial therapy (Barzilai et al. 1999). IL-1β also correlates with the age of the children in the way that younger children have higher levels of IL-1β than older ones (Yellon et al. 1991, Juhn et al. 1992).

IL-1β, IL-6 and TNF-α concentrations measured from the nasopharyngeal secretions of RAOM children have been lower compared to healthy children (Lindberg et al. 1994). It has also been postulated that RAOM children may have a low IL-2 production leading to lower sIgA levels in mucosal secretions (Bernstein et al. 1993b). On the other hand, inflammatory cytokines can also enhance bacterial adherence and invasion to cells (Cundell et al. 1995, Tong et al. 1999), and hence, if a respiratory virus infection, which usually precedes an AOM attack, induces a too high or a prolonged cytokine production, this might theoretically increase the risk of developing AOM.

Human immunoresponse against otitis pathogens

In general, both systemic and local immune reactions play a role in the pathogenesis of AOM. The pathogenesis is dependent on many factors including the virulence of causative microbes, anatomical structures, physiological conditions and individual's capability to set going adequate immunoresponse. This is further dependent on the age of the child (younger ones have a weaker antibody response), genetically determined immunological capacity and on the antigenic stimulus of the organism (Bluestone and Klein 2001).

Summary of the pathogenesis of acute otitis media

In summary, the development of AOM requires pathogens to adhere to the nasopharyngeal epithelium; they must be able to enter the middle-ear cavity through the ET, and, after entering they must be able to overcome the defensive mechanism of the middle-ear. The pathogenesis of AOM is multifactorial and complex, and it is affected by many external and internal factors including normal flora, bacterial-viral interactions and individual immunological reactions. A viral respiratory track infection initiates the process causing congestion of mucosa and leading to the obstruction of the ET and negative middle-ear pressure. It also promotes adherence of bacteria in the

inflamed epithelium by destroying the normal mucociliary defence mechanism of the ET and the middle-ear, thus facilitating pathogenic bacteria to enter the middle-ear. Individual inflammatory reactions and anatomical considerations with micro-ecology of the nasopharyngeal bacterial flora are probably the most important factors to determine whether the final outcome is AOM.

3. Alteration in the epidemiology and treatment

Occurrence

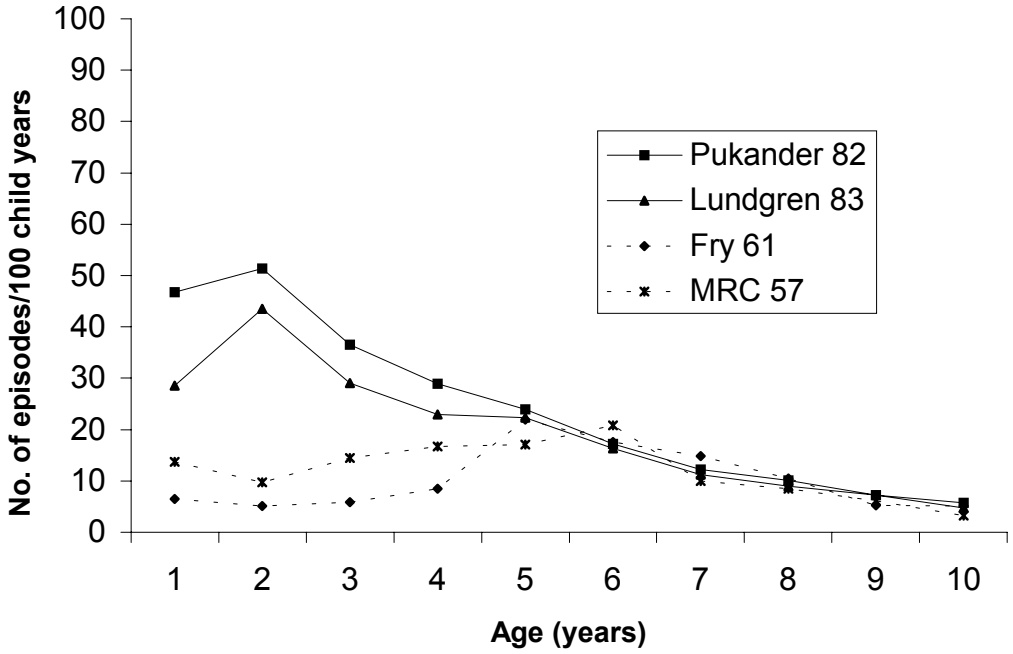
There is a growing body of evidence indicating that the occurrence of AOM is increasing. However, this has been difficult to prove since the differences in the definition of AOM as well as in the study designs contribute to the results of epidemiological surveys made in different years. Nevertheless, there is some data to support the conception that AOM has become a more common, in particular among small children:

1. In general, older epidemiological surveys tend to report lower incidence figures than more recent ones. Fry (1961) reported 778 AOM attacks affecting 333 children under 10 years of age during a 10-year observation-period. Since there were 786 children at risk, the incidence rate was 10 per 100 child-years. This was close to the 12 per 100 child-years reported by the Medical Research Council (1957) in children under 10 years of age. In subsequent population based surveys conducted in the late 1970's, two times higher incidence rates (range from 19 to 25 per a 100 child-years) were observed in the same age group (Pukander et al. 1982, Lundgren and Ingvarsson 1983) (Figure 3). More recent, cohort based surveys have reported even higher figures (Sipilä et al. 1987, Teele et al. 1989, Alho et al. 1991b) (Figure 4). Recently, data from Finnish Otitis Media Cohort Study revealed the incidence rate of 140 per 100 child-years in children under 24 months of age (Kilpi et al. 2001).

2. Time-to-time surveys from the US have reported an increasing out-patient visit trend for OM: According to a national survey of office diagnoses from 1975 through 1990, annual visit rates for otitis media more than doubled; for children under 15 years, the rates increased by 175% (Schappert 1992). McCaig and Hughes (1995) investigated changes in oral antimicrobial prescribing for otitis media by 1980 through 1992 and found increasing antibiotic prescription rates and an increasing trend in visits for otitis media. Lanphear et al. (1997) reported the results of an interview survey of

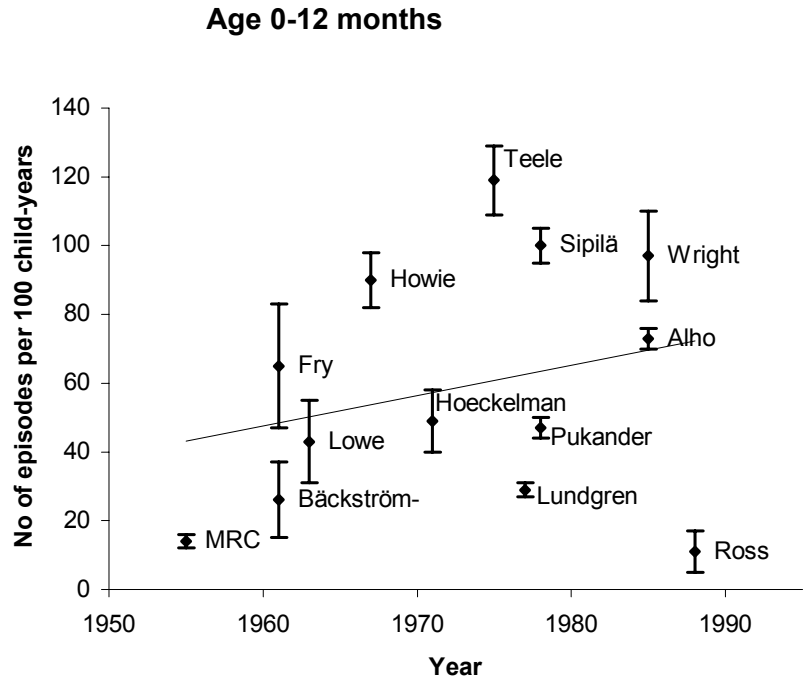
the occurrence of recurrent otitis media among approx 6000 children under 5 years in 1981 and 1988: A history of recurrent otitis was obtained by an affirmative response to the question, “Has your child ever had frequent or repeated ear infections?”. On the basis of this questionnaire, the authors reported a 43.9% rise in the occurrence of RAOM.

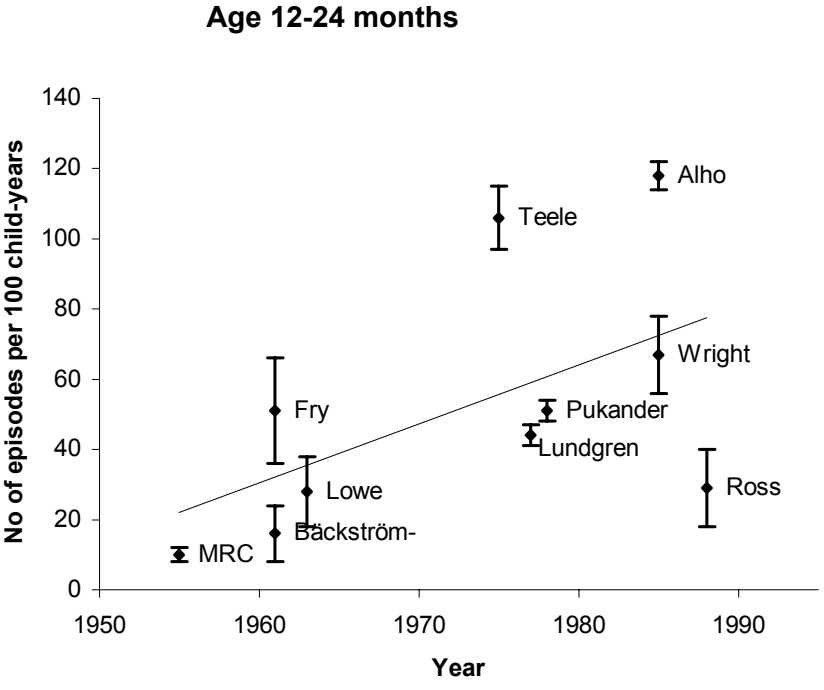
Figure 3. Occurrence of AOM according to Medical Research Council (MRC, 1957), Fry (1961), Pukander et al. (1982) and Lundgren and Ingvarsson (1983)



However, the differences in diagnostic criteria and factors like the parents’ awareness of AOM, makes it difficult to compare incidence data from different time periods. In addition, the differences in study designs and demography, and factors like the availability of healthcare, may have contributed to an apparent change in AOM occurrence.

Figure 4. Occurrence of AOM in children as reported in literature (Medical Research Council (MRC) 1957, Fry 1961, Lowe et al. 1963, Bäckström-Järvinen et al. 1966, Howie et al. 1975, Hoekelman 1977, Pukander et al. 1982, Lundgren and Ingvarsson 1983, Wright et al. 1985, Sipilä et al. 1987, Ross et al. 1988, Teele et al. 1989, Alho et al. 1991b). The marks represent point estimates and 95% confidence intervals for incidence rate. "Year" refers to the first data-collecting year, if mentioned, otherwise it refers to the publication year.





Age distribution

In the last few decades, a shift in the age distribution of AOM seems to have taken place: In the surveys by Medical Research Council (1957), Fry (1961) and Paavolainen (1966) the occurrence of AOM peaked at 3-6 years of age while in the more recent studies the highest occurrence has been found among children under 2 years of age (Pukander et al. 1982, Lundgren and Ingvarsson 1983, Teele et al. 1989) (Figure 3).

Since AOM is frequently a self-limited disease, it is not clear if this change is true or due to biasing factors like the parents' consciousness of this disease. For example, in the surveys by Medical Research Council (1957) and Fry (1961) otorrhea accompanied AOM in 42% to 56% among infants but only in 27% to 33% among children aged over 2 years. This can be due to a true difference in the clinical picture of AOM in different age groups, but it can also reflect a trend to seek medical attention with infants only when the ear becomes discharging. Since older children can usually complain about ear pain, they might be taken to a physician more easily. Therefore, the "true" occurrence of AOM in infants in 1950's might have been higher than it was actually registered. However, a higher occurrence of acute otorrhea in a younger age group was observed in a later survey as well (Pukander 1983). In conclusion, current data is persuasive to the idea that an increasing number of younger children contract AOM than in the 1950's.

Clinical picture

Acute otorrhea due to spontaneous perforation of eardrum sometimes occurs during AOM. It can be considered as a sign of a more aggressive infection in the middle-ear cleft. According to reports from Western countries in the 1950's and in the 1960's, 30-40% of the patients with AOM were accompanied by a discharge from the ear (Fry

1961, Medical Research Council 1957), while in later studies lower figures (4.6% to 17%) are generally reported (Pukander 1983, Andersen et al. 1997).

Serious complications of AOM (mastoiditis, meningitis, brain abscess) are very uncommon these days. In the preantibiotic era, however, acute mastoiditis was the most common infection for which children were hospitalised (Bluestone and Klein 2001). Along with the introduction of antimicrobials (sulfonamides in 1936 and penicillin in 1942) a dramatic decrease took place in the incidence of complications of AOM. At the Otolaryngological Hospital of the University of Helsinki, in the period from 1930 to 1960 there were 99 cases of otogenic brain abscess whereas from 1961 to 1969 there were only 3 cases (Tarkkanen and Kohonen 1970). Currently, fewer than 0.01% of the patients with AOM develop acute surgical mastoiditis in Western countries (Palva et al. 1985). Since in the 1950's and 1960's mastoiditis still occurred in 0.1-0.4% of patients (Medical Research Council 1957, Fry 1961, Paavolainen 1966), it seems likely that the antimicrobial treatment does not completely explain the decrease in the incidence of serious complications of AOM.

Group A streptococcus (GAS) is a common cause of acute mastoiditis (Table 3). Because the incidence of this pathogen as a cause of AOM has decreased (Table 2, p. 16 and Table 4, p. 39), another possible explanation for the decreasing rate of acute mastoiditis are microbiological alterations - particularly a decreasing incidence of GAS infections. Probably a decreasing virulence of other otitis pathogens has affected this phenomenon as well.

Alho et al. (1997) studied surgical cases of both chronic suppurative otitis media and cholesteatoma in Finland and found that the number of new cases has declined markedly. The authors suggested that a possible reason for this is the introduction of antimicrobial drugs in the treatment of OM.

All these data support the concept that AOM is now a milder infection than in the past. Because of the low risk of complications and the high spontaneous resolution rate, AOM is currently considered to be a trivial infection. However, the recent emergence of resistant bacteria and the changes in the virulence of otitis pathogens can still increase the occurrence of serious complications (Antonelli et al. 1999).

Table 3. Relative commonness of bacterial pathogens in acute otitis media (AOM), secretory otitis media (SOM), chronic otitis media (COM) and in acute mastoiditis (+++ most common isolate, ++ common, + uncommon, - very uncommon or does no exist)

	AOM	SOM	COM	Mastoiditis
Streptococcus pneumoniae	+++	++	+	+++
Haemophilus influenzae	+++	+++	+	+
Moraxella catarrhalis	+++	++	-	-
Group A Streptococcus	+	-	-	++
Stafylococcus aureus	+	+	++	+
Pseudomonas aeruginosa	-		+++	+

No growth: AOM 7-39%, SOM 34-66%, Mastoiditis 16%
Data modified from (Linder et al. 2000, Bluestone and Klein 2001, Kilpi et al. 2001)

Alteration in treatment

In the US, McCaig and Hughes (1995) addressed changes in oral antimicrobial drug prescribing by office-based physicians. Data was collected from the National Ambulatory Medical Care Survey from 1980 through 1992. For OM, it was found that the prescription of more expensive, broad-spectrum antibiotics, such as the cephalosporins and amoxicillin, had increased.

Two studies from England reported increasing otitis media related surgery trends: Friedman et al. (1989) analysed data relating to operations for SOM in children under 10 years of age during the period of 1975-1984. The annual surgery rate for SOM rose from 45/10000 to 107/10000 representing an increase of 137%. Black (1984) made an analysis based on hospital statistics in the Oxford region revealing a 74% rise of surgical rates for OM between 1975 and 1981. Similar trends have also been reported elsewhere: In Finland, a nationwide questionnaire survey of OM surgery showed that the number of adenoidectomies performed on children before the age of 3 years

increased by 30% after 1990 (Niemelä et al. 1998). This increase was closely related to increasing day care attendance of children (with increasing occurrence of AOM as a sequels). Increasing surgery trends could be the result of changing practice patterns (changes in the indications for surgery, an easier accessibility of medical services) and a change in the clinical picture of AOM (an increasing number of children with prolonged effusion, increasing recurrence rates).

4. Genetics of otitis media

The susceptibility to many infectious diseases is mediated at least partly by genetic factors. A classical example of this is that persons who are heterozygotes to sickle cell alleles are relatively protected from malaria (Flint et al. 1993). Genetic factors also modify the clinical picture and the outcome of infectious diseases. An example of this is that persons who are homozygotes for TNF allele 2, have a seven times increased relative risk for death or severe neurological sequel due to cerebral malaria (McGuire et al. 1994). Similarly the outcome of meningococcal meningitis and Epstein-Barr virus infection, for example, has been associated with genetic factors (Westendorp et al. 1997, Helminen et al. 1999).

OM is an extremely common disease affecting almost every child, but only approximately 10 to 20 percent of children contract recurrent episodes of OM and are classified as otitis-prone (Alho et al. 1991a). RAOM is a disease with a multifactorial etiology including various environmental and infectious etiologies. There are also several lines of evidence that the susceptibility to RAOM is in part genetically determined. This evidence comes from epidemiological data and twin studies especially, but is also supported by some immunological studies.

Familial aggregation of otitis media

A number of epidemiological studies have shown a familial predisposition of OM. According to the study by Sipilä et al. (1988), the second important risk factor for AOM was a sibling with OM. In a retrospective study, Stenström and Ingvarsson (1997) compared otitis-prone children with age and sex matched controls and found that more of the otitis-prone children had fathers who had been otitis-prone as children and it was more often the case that the otitis-prone group had siblings who were also otitis-prone than the controls. In the Greater Boston study, the risk for AOM increased

significantly if the child had a sibling with a history of RAOM (Teele et al. 1989). A meta-analytic review of risk factors identified a positive family history of AOM as the most important risk factor for AOM, the relative risk being 2.6 (Uhari et al. 1996).

Racial differences

Relatively high incidences of OM have been reported in certain ethnic groups, like in American Indians, Eskimos and Australian Aborgines (Wiet et al. 1980, Leach 1999, Curns et al. 2002). As an example outpatient OM-associated visits among American Indian and Alaska Eskimo children were twice as frequent as those among Caucasian children (Curns et al. 2002). In addition to the high occurrence of AOM among these ethnic groups, their ear-infections are also more complicated and chronic otitis media is a common sequel (Bluestone 1998). Probably many external factors contribute to this, including overcrowded households, maternal smoking, and poor hygiene (Curns et al. 2002, Coates et al. 2002). However, the occurrence of OM among Apache children who were adopted into non-Apache homes out of the reservation did not differ from that among Apache children remaining on the reservation (Spivey and Hirschhorn 1977). Consequently, genetic factors seem to be important in explaining these racial differences.

Sex differences

Males tend to have a higher occurrence of OM than females (Pukander et al. 1982, Sipilä et al. 1988, Teele et al. 1989, Alho et al. 1990, Kilpi et al. 2001). Recently Kilpi et al (2001) found that the higher incidence of AOM among boys is especially attributed to an increasing number of infections caused by *H. influenzae* and recurrent AOM. The basic reason for male-related increasing susceptibility to AOM is not recognized, but this may be associated with genetically determined anatomic or physiological variability. Perhaps the rougher male play behaviour causes heavier exposure to environmental risk factors.

Twin Studies

In the retrospective study of 2750 pairs of adult Norwegian twins, the occurrence of recurrent ear infections was estimated according to an affirmative answer to the question: "Do you have, or have you had recurrent infections in the ear?" (Kvaerner et al. 1997). Accordingly, 8.9% of subjects gave positive answer with a significant predominance of females (10.6%) compared to males (6.9%). The role of heritability was estimated at 74% for females and 45% for males. The weakness of this study was its retrospective design which relied on self-reporting of OM frequency. For example, respondents who reported a positive history of ear infections could have had either AOM or SOM. It is also noteworthy that the mean age at the onset of self-reported ear infections was 2.7 years, which is higher than it is generally observed by other investigators (Pukander et al. 1982, Lundgren and Ingvarsson 1983, Teele et al. 1989). Finally, the female predominance in the occurrence of ear infections is not supported by many other surveys (Pukander et al. 1982, Sipilä et al. 1988, Teele et al. 1989, Alho et al. 1990, Kilpi et al. 2001). This study may, thus, have been affected by a diagnostic bias.

The most convincing evidence of a genetic component involved in middle-ear infections comes from a prospective twin/triplet study from Pittsburgh, US (Casselbrant et al. 1999). Investigators enrolled altogether 168 twins and 7 triplets in the study within their first 2 months of life. The children were followed up by regular visits to the study clinic. The follow-up time was 2 years. The discordance estimate for recurrent episodes of AOM was 0.04 for monozygotic and 0.49 for dizygotic twins/triplets. The correlation of the proportion of time with MEE ranged from 0.34 to 0.39 in dizygotic sets and 0.63 to 0.83 in monozygotic sets. The estimated heritability of OM was 64% for males and 79% for females (the difference between males and females was not statistically significant).

Recently, a large population cohort of all twins born in England and Wales in 1994, was studied prospectively at the ages of 2, 3, and 4 years (Rovers et al. 2002). According to questionnaire data, the genetic influence for the symptoms of earache at

ages 2, 3 and 4 was 58%, 39% and 60%, respectively. Unfortunately, the questionnaire used in this study was rough, and, therefore, the results should be interpreted critically.

Anatomic studies

Beery et al. (1980) studied the ET function in American Indians and compared the results with the Caucasians. The ET of the American Indians had a lower passive tubal resistance, and thereby ET's were functionally different from those of the Caucasians. It is not clear, however, if this was due to a real anatomic difference between the races or secondary to an ET inflammation process.

Due to the fact that RAOM is most common among small children and as children have a shorter and a straighter ET than adults (Figure 2), it has been logical to investigate the differences in the nasopharyngeal anatomy in subjects with a high occurrence of a middle-ear disease. A Finnish study analysed the occurrence of acute otitis media in 238 schoolchildren who were X-rayed for orthodontic purposes (Niemelä et al. 1994). The means for almost all the measured dimensions of the bony nasopharynx were significantly smaller in the children with two or more attacks of AOM in their history than in those with fewer than two attacks. Consequently, genes controlling the anatomy of the skull bone might be involved in RAOM.

Immunoglobulin secretion

The children with RAOM have been shown to have lower plasma concentrations of IgG subclass than children who are not otitis prone (Freijd et al. 1984, Freijd et al. 1985). The occurrence of certain immunoglobulin allotypes has been shown to correlate to the concentrations of these subclasses: Mean concentrations of IgG2 and IgG4 were higher in adults who had a genetic marker Gm(23) (Morell et al. 1972). Based on these observations, a Swedish group studied genetically determined immunoglobulin markers in 20 families, each comprising of a parent and a child, both

with a history of RAOM (Prellner et al. 1985). The control group was 47 children with no episodes of AOM. Surprisingly, 95% of the RAOM children had the IgG2 marker G2m(23), whereas the marker was found in 64% among the control group the difference being statistically significant (Prellner et al. 1985, Kelly 1993). Consequently, the heredity of immunoglobulin secretion seems to be a complex phenomenon and assumptions made by studies conducted with adults do not necessary work with children.

Siber et al. (1990) studied the immunoresponse to *H. influenzae* polysaccharide vaccines among healthy Apaches. Even though the total IgG, IgM, and IgA levels were higher in 24-month-old Apache children than in white children, the concentrations of *H. influenzae* type b antibody were approximately 10-fold lower after immunization with a polysaccharide vaccine. Moreover, IgG2 and IgG4 subclasses were lower among Apache children indicating that hereditary immune deficiency may explain racial differences of susceptibility to AOM.

Recently, more proof was evinced of the genetically determined low immunoglobulin secretion and RAOM. Ninety-four percent of RAOM children who had a positive sibling history of RAOM had inadequate IgG2 antibody titres against the four most common pneumococcal serotypes (6B, 9V, 19F and 23F), compared to 76% of RAOM children without a positive sibling history (Damoiseaux et al. 2000).

HLA-studies

Kalm et al. (1991) et al compared the frequencies of certain HLA antigens between 45 children with RAOM and controls. RAOM was defined as six or more episodes of AOM during a 12-month period. HLA-A2 antigen was found in 36/45 (80%) of the subjects with RAOM, as compared with 951/1701 (56%) “apparently healthy individuals” (RR = 3.15) and 10/22 (45%) of prospectively followed children without any AOM episodes. However, in another study, they did not find this association in individuals with SOM suggesting a divergent influence of the heredity on the

pathogenesis of RAOM and SOM (Kalm et al. 1994). The results of these two studies have not been confirmed elsewhere.

Surfactant protein-A

Surfactant protein-A, which plays a role in innate host defence in the lung, is also expressed in the ET (Paananen et al. 2001). Recently Rämet et al. (2001) from Oulu, Finland, studied surfactant protein-A genetics among 147 children, admitted to hospital for adenoidectomy and/or tympanostomy with at least 5 episodes of AOM by the age of 1 to 10 years (RAOM group). The controls were 278 infants from the same district, but the occurrence of AOM in the control population was not known. A significant difference ($p=0.04$) in the frequency of surfactant protein haplotypes was noticed. This was mainly due to an over-presentation of haplotype $6A^4$-$1A^5$, which occurred in 10.2% of the cases in RAOM compared to 6.1% in the control group. The clinical significance of this finding has not yet been demonstrated.

Mannan-binding lectin

Some children, suffering from recurrent upper-airway infections and diarrhea during infancy, have been shown to have a common complement-dependent opsonic defect, caused by a low concentration of mannan-binding lectin (protein) (Super et al. 1989). A certain allelic variant of mannan-binding lectin has been associated with low concentrations of this protein. Two studies compared certain genotypes of the mannan-binding lectin gene between children with RAOM and controls but have not come up with any association (Garred et al. 1993, Homoe et al. 1999).

Cytokine genetics

Recently, cytokine genes were implicated as candidate genes in OM (Casselbrant and Mandel 2001). The production of cytokines is regulated by genetic elements. Like most human genes, cytokine genes are polymorphic. Polymorphism associated with these genes is thought to lead to individual variations in cytokine secretions, and it is inherited in a Mendelian fashion (Hurme et al. 1998). The outcome of various infectious diseases, such as malaria, meningococcal meningitis, and Epstein-Barr virus infections, has been associated with this polymorphism (McGuire et al. 1994, Westendorp et al. 1997, Helminen et al. 1999).

5. Microbiological trends

S. pneumonia is the most common single isolate responsible for AOM accounting for one third of the cases on the average. This is followed closely by *H. influenzae* and *M. catarrhalis* which are responsible for one fifth of the cases each (Table 2, p. 16). However, before 1980's, *M. catarrhalis* was an infrequent finding in AOM, and it was considered quite apathogenic. In the early 1980's a Finnish study group proved preliminary serologic evidence for a pathogenic role of *Moraxella catarrhalis* in AOM (Leinonen et al. 1981). In Dallas, Texas, bacteriological studies of AOM carried out between 1973 and 1986 revealed a steady rise from 2.0% to 25.0% in the occurrence of *M. catarrhalis* isolates (Nelson 1986). During this period the patient population did not change much demographically and the methods for obtaining middle-ear fluid, performing cultures and identifying micro-organism were uniform. A similar trend in the increase in the occurrence of *M. catarrhalis* in AOM has been observed in Finland as well. The proportion of *M. catarrhalis* AOM more than doubled between three studies conducted 20 years apart (Luotonen et al. 1981, Karma et al. 1985, Kilpi et al. 2001). The changes in the microbiology can sometimes be very rapid: In a prospective study Shurin et al. (1983) could isolate *M. catarrhalis* in MEE in 6.4% of children between 1979 and 1980. Between 1980 and 1982 *M. catarrhalis* was isolated in 26.5% of children. No explanation could be presented for this dramatic increase. These observations indicate that *M. catarrhalis,* which was long thought to be a non-pathogenic bacterium, has acquired virulence properties.

A second prominent change that has occurred is a decreasing incidence of group A streptococcus (GAS) isolates in AOM. While in early studies it was detected in up to 24% of MEE, in later studies it has been an infrequent cause of AOM (Table 2, p. 16). However, a transient epidemic of GAS otitis can still occur (Jensen and Ejlertsen 1990). Along with decreasing incidence, GAS has also become less virulent in recent decades, and manifests usually as a mild pharyngitis (Stollerman 2001).

Alterations in the incidence of *S. pneumoniae* isolates have been observed as well. Bluestone et al. (1992) reported a significant rise in the prevalence of pneumococcus in children with AOM during the 1980s. In 1980, this organism was cultured in 28% of effusions, compared with 44% in 1989. The reason for this increase is unclear, and this kind of trend has not been reported elsewhere.

The rates of antimicrobial resistance in bacteria responsible for AOM have been alarmingly increasing. In a study carried out in the microbiology laboratory of Tampere University Hospital, Finland, the first β-lactamase producing *M. catarrhalis* strains were detected in 1979 (Nissinen et al. 1995a). The prevalence of such strains increased rapidly reaching 57% in 1983 and 80% in 1990 Since then β-lactamase production of *M. catarrhalis* has still increased and currently only 4-6% of the isolates do not produce β-lactamase (Manninen et al. 1997, Kilpi et al. 2001). This trend is consistent with trends elsewhere in Scandinavia as well as in the US (Olsson-Liljequist et al. 1992, Barry et al. 1994).

β-lactamase production among isolates of *H. influenzae* has also increased but less rapidly. Currently 14-24% of *H. influenzae* isolates in Finland (Kilpi et al. 2001) and 41.6% in the US produce β-lactamase (Manninen et al. 1997, Jacobs et al. 1998, Kilpi et al. 2001).

The emergence of penicillin resistant *S. pneumonia* is the most alarming alteration in antimicrobial resistance of otitis pathogens, due to a high prevalence of this microbe as a cause of respiratory track infections, and as pneumococcal otitis can be severe and lead to complications (Antonelli et al. 1999). In addition, AOM attacks caused by *S. pneumonia* are less likely to have a spontaneous resolution, than episodes caused by *H. influenzae* (Howie and Ploussard 1972) and probably *M. catarrhalis*.

In Spain, the proportion of penicillin-resistant pneumococci rose rapidly from 6% in 1979 to 44% in 1989 (Fenoll et al. 1991). In general, the highest rates of non-susceptible pneumococci have been reported in countries where antibiotics are freely available - for example Taiwan with the rate of 91.3% (Lee et al. 2001), and the lowest rates in countries with a restrictive antibiotic policy - in the Netherlands, for example, with the rate about 1% (Hermans et al. 1997). Fortunately, the occurrence of non-

susceptible *S. pneumoniae* strains in Finland has been reported to be relatively low, 4-5%, so far (Manninen et al. 1997, Kilpi et al. 2001).

In conclusion, according to the studies referred above the microbiology of AOM is now different than two-three decades ago (Table 4). The increasing incidence of *M. catarrhalis* in OM in children is one of the most prominent alterations. It also seems possible that this bacteria has become more virulent. Instead, otitis media due to GAS has become infrequent and less virulent. The antimicrobial resistance of middle-ear pathogens has emerged alarmingly over time.

Finally, our understanding of negative bacterial cultures obtained from MEE has recently changed. While previously these effusions were thought to be sterile it has become clear that even negative cultures can contain metabolically active bacteria detected by the presence of the bacterial messenger RNA (Rayner et al. 1998).

Table 4. Summary of microbiological changes in AOM

Bacteria	Change
Streptococcus pneumoniae	non-susceptible strains ↑
Haemophilus influenzae	β-lactamase producing ↑
Moraxella catarrhalis	β-lactamase producing ↑, incidence ↑, virulence ↑ (?)
Group A Streptococcus	Incidence ↓, virulence ↓

Purpose of the study

1. To find out about the potential changes in the epidemiology of acute otitis media (AOM) during the past two decades

2. To evaluate the changes in the clinical picture of AOM

3. To evaluate the alterations in the treatment of AOM

4. To study cytokine gene polymorphism in recurrent acute otitis media (RAOM)

5. To study the occurrence of resistant bacterial pathogens in the nasopharynx of children with RAOM

Subjects and methods

Alteration in the epidemiology, clinical picture and treatment (I and II)

The study was retrospective, cross-sectional and population based. All episodes of AOM in children under ten years of age diagnosed by a physician in two 12-month periods in Kurikka and Jalasjärvi, were registered. The clinical picture, the given initial treatment, the complications and the surgical procedures were also registered. The first study period was from June 1, 1978 to May 31, 1979, and the latter from June 1, 1994 to May 31, 1995.

The total population in the study area was about 21 000. Out of the total population 2921 in 1978 and 2611 in 1994 were under 10 years of age, and 51% and 53% were boys, respectively. The number of children under 6 years of age was 1701 in 1978-79 and 1551 in 1994-95.

The data were collected from all available medical files in the area. Most AOM episodes were treated in primary health care centres by general practitioners, but the registration was also compiled by relevant private medical services and local hospitals. The data collecting methods were the same in both study periods.

The primary physicians' diagnoses were checked and confirmed by reviewing the case records. For a diagnosis of AOM, there had to be both acute symptoms (at least one of the following: earache, ear rubbing, restless sleep, irritability, fever or other acute respiratory symptoms) and suggestive otoscopic signs (distinct redness and/or outward bulging of the tympanic membrane or, by pneumatic otoscopy, suspicion of effusion). Acute otorrhea alone (through a spontaneously perforated tympanic membrane) also qualified for the diagnosis. There had to be a minimum time-period of 30 days between separate attacks, unless the patient's ear had already been judged as cured by a physician.

Cytokine gene polymorphism (III)

The study was a case-control study and it was carried out at Tampere University Hospital between July 1997 and January 2000. Twenty different families with a high occurrence of AOM were enrolled in the study. In each family, at least one of the parents and all the children had a history of RAOM. The criteria for RAOM were at least 6 AOM episodes within a 12-month period or altogether at least 10 AOM episodes during a lifetime. The medical history was based both on medical records and on an interview.

A blood sample for the genetic analysis was obtained from 63 RAOM subjects. Control samples were obtained from 400 unselected healthy blood donors (The Finnish Red Cross Blood Transfusion Centre, Tampere) living in the same area as the study group members. The donors were 18-60 years of age. The history of AOM in the control population was not known.

The distribution of the TNF-α (-308), IL-1α (-889), IL-1β (-511), IL-1β (+3953) and IL-1Ra gene allele frequencies in the RAOM families were compared with healthy blood donors.

DNA was prepared by standard methods from citrated whole blood.

TNF-α (-308): A 107-bp fragment of the TNF-α gene promonter region containing the G-to-A substitution was amplified using PCR (polymerase chain reaction) primers: 5'-AGGCAA TAGGT TTTGA GGGCCAT-3'and 5'-TCCTC CCTGC TCCGA TTCCG-3'. The amplified product was digested with NcoI and analysed with electrophoresis on a 9% polyacrylamide gel (PAGE) (Wilson et al. 1992).

IL-1α (-889): The base exchange at the position –889 of the IL-1-α gene was analysed as previously described (McDowell et al. 1995). Oligonucleotides 5'-AAGCTTGTT CTACC ACCTG AACTAGGC-3' and 5'-TTACA TATGAGC CTTCCATG-3' flankin the polymorphic site were used as primers in PCR. The products were digested with NcoI and the resultant products were analysed on 9% PAGE.

IL-1β (-511): The region that contains the AvaI polymorphic site at the position –511 of the IL-1-β gene was amplified by PCR. The oligonucleotides 5'-TGGCA TTGAT

CTGGT TCATC-3' and 5'-GTTTA GGAAT CTTCC CACTT-3' were used as primers. Fragments were analysed by electrophoresis on 9% PAGE, stained with ethidium bromide (di Giovine et al. 1992).

IL-1β (+3953): Position +3953 within exon 5 of the IL-1-β gene has a single base-pair polymorphism (Pociot et al. 1992). The polymorphic region containing the TaqI restriction site was amplified using the following primers: 5'-GTTGT CATCA GACTT TGACC-3' and 5'-TTCAG TTCAT ATGGACCAGA-3'. Fragments were analysed after electrophoresis on 9% PAGE, stained with ethidium bromide.

The IL1Ra exon 2 polymorphism was analysed as described (Tarlow et al. 1993). Oligonucleotides 5'-CTCAG CAACAC TCCTAT-3' and 5'-TCCTG GTCTG CAGGTAA-3' were used as primers in PCR. The final PCR product was analysed by electrophoresis on a 2% agarose gel stained with ethidium bromide.

Nasopharyngeal carriage of resistant bacteria (IV)

The study design was cross-sectional. From October 1996 to February 1999 nasopharyngeal bacterial cultures were taken from 306 children who had suffered from RAOM and were undergoing ventilation tube insertion and/or adenoidectomy in the Department of Otolaryngology, Tampere University Hospital, Finland. They belonged to a group of 2497 children initially enrolled in the Finnish Otitis Media Vaccine Trial at the age of 2 months (Eskola et al. 2001).

In these children, each AOM episode had been treated with antibiotics - amoxicillin being the first-choice drug. Those allergic to amoxicillin received trimethoprim-sulfadiazine, cefaclor or azithromycin. According to vaccine trial protocol, antimicrobial prophylaxis (amoxicillin 20 mg/kg or trimethoprim-sulfadiazine 4mg/12.5mg/kg once a day) was started after 3 to 5 episodes of AOM within 6 months or 4 to 6 episodes within 12 months, and the child was referred for operative treatment (insertion of ventilation tubes and adenoidectomy).

In the operating room, a nasopharyngeal sample for bacterial culture was taken with a cotton-tipped wire swab during anaesthesia at the beginning of the operation. The

sample was immediately placed in a transport medium (Transpocult, Orion Diagnostica, Orion Co., Espoo, Finland) and sent within 6 hours to the microbiological laboratory of Tampere University Hospital.

Culture and susceptibility testing was performed by standard procedures (Performance standards for antimicrobial disk susceptibility tested. 1997). The penicillin susceptibility of *S. pneumoniae* strains was screened by the oxacillin disk method (1 μg; Oxoid Ltd., Basingstoke, Hampshire, England), and the minimal inhibitory concentration (MIC) was determined by the penicillin E-test when appropriate (AB Biodisk, Solna, Sweden). MIC values <0.06 μg/ml were defined as susceptible (S), 0.12-1.0 μg/ml as intermediately resistant (I) and ≥2μg/ml as resistant (R).

Data processing and statistical methods

Data processing was made by SPSSWin (version 6.0, SPSS Inc., Chicago, Illinois) and Statistica (version Win5.1, StatSoft Inc, Tulsa, OK, USA) software. Age specific (from 0 to 10 years) incidence figures were based on the annual census data of population in Finland (Official Statistics of Finland. Population 1978 and 1994). Fisher's exact or chi-square test, when appropriate, was used to compare the results of the microbiological culture and susceptibility testing and the distribution of the cytokine gene allele frequencies and to study the differences between the incidence figures of AOM between 1978-79 and 1994-95. A Stepwise logistic regression analysis was used to study the parameters associated with recurrent attacks. Odd ratios (OR) and 95% confidence intervals were calculated using CIA software (version 1.1, copyrighted by MJ Gardner and British Medical Journal, 1989).

Ethics

Permission for the epidemiological part of investigation (I and II) was obtained from the Ministry of Health and Social Welfare of Finland. Other studies (III, IV) were approved by the Ethical Committee of Tampere University Hospital and a written informed consent was received from the parents.

Results

Occurrence and recurrence of acute otitis media in 1978-79 vs. 1994-95 (I)

In 1978-79 420 and in 1994-95, 550 children under 10 years of age suffered from at least one AOM episode during the study period (14% and 21% of the reference population, respectively). The total number of AOM episodes was 566 in 1978-79 and 835 in 1994-95, and the incidence rates were 19 and 32 per 100 child-years, respectively. The increase in the occurrence was 68% (95% CI 53-75%) (Figure 5 a).

The occurrence of AOM rose especially among boys with the increase 85% (Figure 5 b). Among girls the increase was 42%. The highest rate in 1994-95 was found among children aged 12-24 months (rate 91 among boys and 39 among girls per 100 child-years, Figure 5 b).

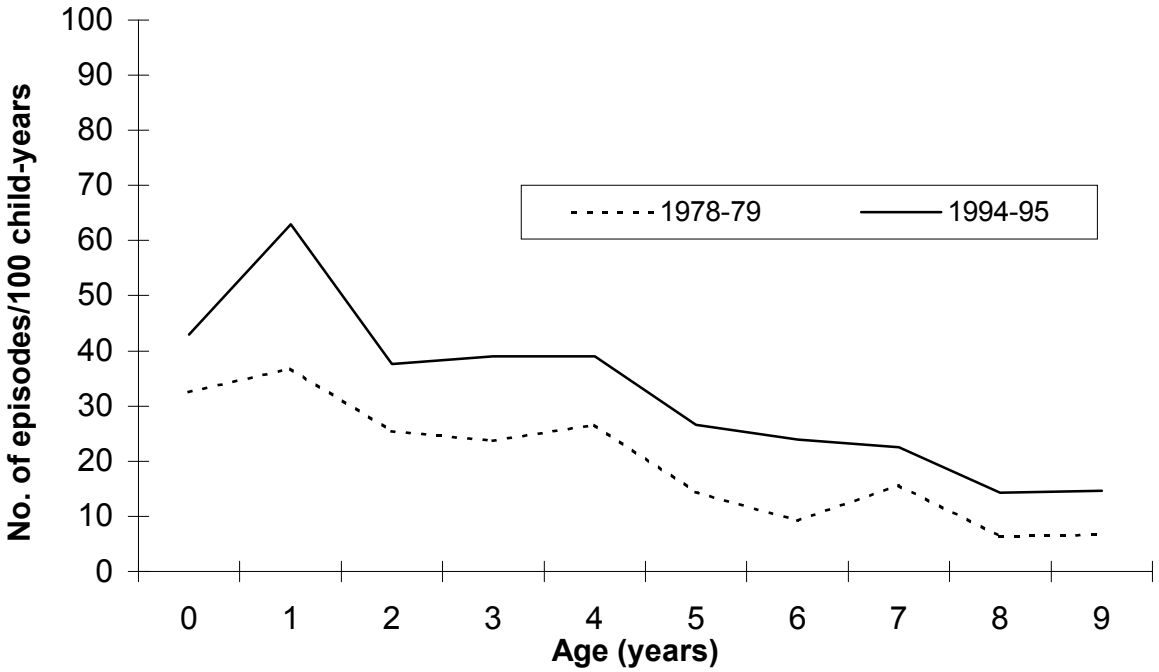
The number of recurrent attacks increased markedly. In 1978-79 6% and in 1994-95 13% of the children with AOM during the study year had at least two recurrent episodes (OR = 2.3, 95% CI 1.4, 3.8). At least three recurrences experienced 2% and 6% respectively (OR = 3.7, 95% CI 1.6-8.5). Recurrences (any) were the most common among children aged 12-24 months.

Symptoms, treatment and sequels of acute otitis media in 1978-79 vs. 1994-95 (II)

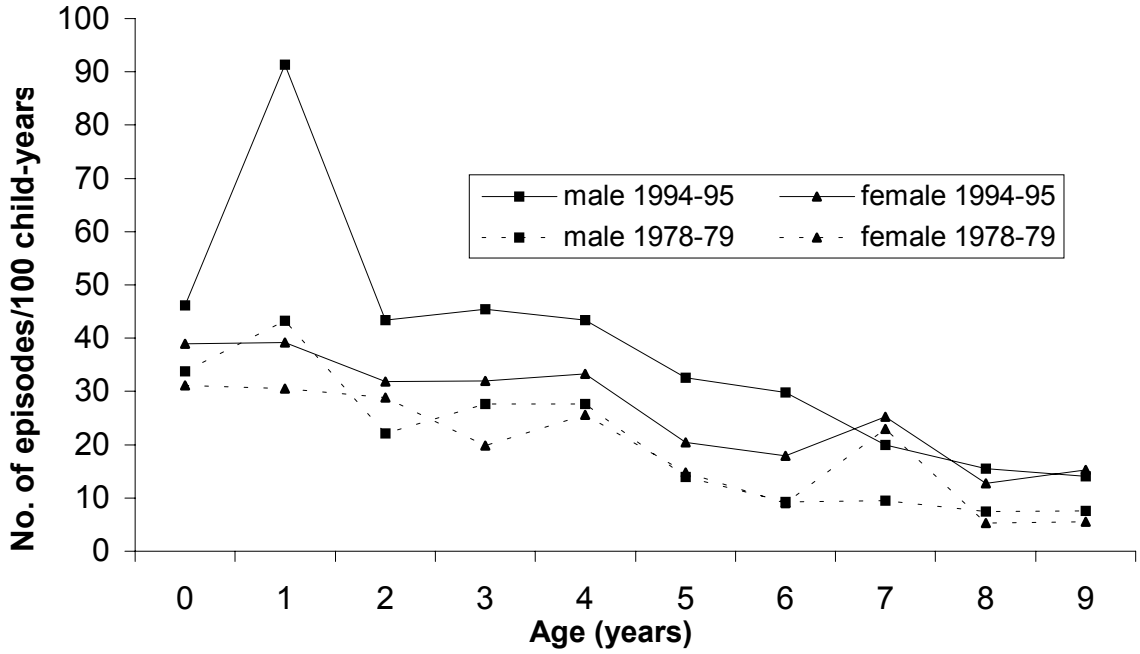
The time delay from the onset of the otitis suggesting symptoms (ear-rubbing, irritability, restless sleep, aural pain) to an office visit with the AOM diagnosis was shorter and proportion of febrile patients was lower in 1994-95 than in 1978-79 (Table 5). Moreover acute otorrhea (through spontaneously perforated eardrum) occurred less frequently in 1994-95 (Table 5).

Figure 5 a). Occurrence of acute otitis media in 1978-79 and 1994-95. b) Occurrence by sex

a)



b)



Of all the AOM attacks 97.6% in 1978-79 and 98.9% in 1994-95 were treated with antimicrobials. Prescribing penicillin-V, for the initial treatment, decreased heavily in favour of broad-spectrum drugs (Table 5).

In a logistic regression model for one or more recurrences, significant risk factors were initial broad-spectrum antibiotic treatment (OR 1.8, 95% CI 1.3-2.6), young age and a previous otitis. The non-significant predictors in the modelling were the study period, the sex and the tympanocentesis.

The frequency of adenoidectomy and/or tube insertion doubled from 1978-79 to 1994-95 (Table 5). No children experienced any serious complications of AOM (mastoiditis, meningitis, brain abscess) or needed any major aural surgery in either period.

Table 5. Summary of acute otitis media in 1978-79 versus 1994-95 (percentage of children with AOM)

	1978-79 %	1994-95 %	P
Symptoms > 24h	35.5	9.6	<0.001
Otorrhea	6.3	3.3	0.01
Febrile illness	36.0	26.6	0.002
Bilateral acute otitis media	32.4	30.4	ns
Primary treatment with penicillin-V	80.2	10.5	<0.001
Recurrences ≥2	6.0	12.7	0.001
Adenoidectomy/tympanostomy	6.2	12.4	0.001

Results of genetic analysis of families with high occurrence of acute otitis media (III)

Of the 63 family members enrolled in the study 57% were male and 43% female. The families included were extremely prone to otitis (Table 6). Three subjects had also suffered from subacute or chronic mastoiditis, and mastoidectomy had been done in

two cases. Of the study group 18 were adults and 45 children. Seventeen of the patients had a history of allergic disorders (atopic dermatitis, asthma or allergic rhinitis): 10 in two families and 7 in five families.

Table 6. Characteristics of 20 families with high occurrence of acute otitis media

	Adults	**Children**
Individuals	18	45
Mean age, years	35.5 (28-42)	6.2 (1-16)
Total number of otitis related operations*	18 (0-3)	78 (0-11)
History of mastoiditis	2	1
Allergic disorder	4	13

*Adenoidectomy and/or tympanostomy, 4 adults and 3 children had not been operated

The distribution of proinflammatory cytokine alleles, TNF-α (-308), IL-1α (-889), IL-1β (-511), IL-1β (+3953), and anti-inflammatory IL-1Ra alleles did not differ significantly in the RAOM group versus the control group. A trend of deviance was found between RAOM and IL-1β (-511) allele frequencies (p=0.08). By calculating the children's data separately, the result remained basically the same. However, in a subgroup of RAOM patients without a history of any allergic disorders allele frequencies of IL-1α (-889) differed significantly from those of the controls (Table 7). This was mainly attributed to an increasing occurrence of common 1.1 genotype in RAOM group (OR 2.2, 95% CI 1.3-4.1).

Table 7. Genotype distribution of IL-1α (-889) gene in subjects with recurrent acute otitis media (RAOM) vs. controls

Group	**Genotype (%)**			**Allele Frequency**		**P**[1]
	1.1	**1.2**	**2.2**	**Allele 1**	**Allele 2**	
RAOM (n=58)[2]	51.7	44.8	3.4	0.74	0.26	0.12
RAOM without allergy (n=46)	60.9	34.8	4.3	0.78	0.22	0.03
Controls (n=400)	41.8	50.3	8.0	0.67	0.33	-

[1]P-value calculated for allele frequencies in 2x2 table versus controls
[2]genotyping was successful in 58/63 cases

Nasopharyngeal carriage of otitis pathogens and their antimicrobial susceptibility (IV)

Nasopharyngeal cultures were taken from 306 children (178 boys and 128 girls), aged from 9 to 24 months (median 17 months). At least one pathogen was harboured in 89 subjects (29.1%). Altogether, 93% of M. catarrhalis strains and 43% of *H. influenzae* strains produced ß-lactamase. Eight (25%) out of 32 *S. pneumoniae* strains were non-susceptible (I/R) to penicillin. Of these, six case subjects had pneumococcal isolates with MIC values for penicillin between 0.12 and 1.0 µg/ml and two had MIC value 2.0 µg/ml.

At the time of the sample collection, 23 (72%) out of 32 children with nasopharyngeal carriage of *S. pneumoniae* received prophylactic antimicrobial therapy. Five (22%) of these were having amoxicillin and 18 (78%) trimethoprim-sulfadiazine. All children with non-susceptible *S. pneumoniae* were receiving antimicrobial prophylaxis (2 had amoxicillin and 6 trimethoprim-sulfadiazine) compared to 64% (9/14) of those with susceptible pneumococcus alone, and 80% (8/10) of those with ß-lactamase-producing *M. catarrhalis* together with *S. pneumoniae* (p=0.1474).

S. pneumoniae was isolated as a single pathogen in 22, and with *M. catarrhalis* in 10 samples. Of the pneumococcal strains isolated alone, susceptibility to penicillin was reduced (Pen I/R) in 8 (37%), while all strains isolated with ß-lactamase-producing *M. catarrhalis* proved susceptible to penicillin (p=0.0353, Table 8). The statistical significance remained even if the patients without preoperative antibiotic prophylaxis were omitted (p=0.0261).

Table 8. The effect of concomitant nasopharyngeal carriage of ß-lactamase producing *Moraxella catarrhalis* on the occurrence of penicillin (Pen) resistance of *Streptococcus pneumoniae*

	No. of patients with		
	S. pneumoniae	*S. pneumoniae* + *M. catarrhalis*	**Total**
Pen S	14	10	24
Pen I/R	8	0	8
Total	22	10	32

S, susceptible; I, intermediately resistant; R; resistant
Fisher exact, two-tailed p=0.0353

Discussion

Alteration in the occurrence of AOM (I)

In the present study, from 1978-79 to 1994-95 the total increase in the occurrence of AOM was 68% in children under 10 years of age. Because the proportion of small children in the population decreased between the periods, the age-adjusted total rise would be even higher.

Although the design of the study was retrospective, the data collecting methods and diagnostic criteria used were identical in 1994-95 with those in 1978-79, and the geographical area was the same. There is also no reason to assume that the otoscopic skills of general practitioners have changed during the 15-year period. This finding of increasing occurrences of AOM is also in concordance with surveys from the United States, which have reported increasing out-patient visit rates and increasing antibiotic prescription rates for OM as well as an increasing recurrence rate of OM (Schappert 1992, McCaig and Hughes 1995, Lanphear et al. 1997).

However, since AOM is often a self-limited disease, it is not clear how much of these changes can be explained by factors like parents' consciousness of this disease and a more frequent use of medical services. Earlier visits to physicians in 1994-95 than in 1978-79 show the current trend for an easier consultation (Table 5). Still, it is unlikely that this will completely explain the rise in the occurrence of AOM for several reasons:

1. The trend curve is not equal between males and females (Figure 5 b). Why would the parents of male children have visited a doctor more easily? Rather, it seems that external predisposing factors have changed to an untoward direction, and males are genetically more sensitive to these changes.

2. The heaviest rise was in the number of recurrent attacks, in particular among small children between their first and second birthday. If the change in the occurrence had only been due to an increased use of medical services, one would have expected a more equal rise in all kinds of AOM attacks in all age groups.
3. If the apparent increase in OM detected had entirely been due to increasing diagnostics, over 60% of cases in the period 1978-79 should have gone undiagnosed!
4. If risk factors for AOM in population have changed (see below), the consequence should be change in the occurrence of the disease.

Considering these facts, it seems inconceivable that the rise in the occurrence would only have been due to an increasing use of medical services and other confounding effects.

Reasons for increasing occurrence

Day care

The day-care attendance of each individual child was not registered, but the total number of day-care facilities in the study area increased from 412 in 1979 to 628 in 1995 (52% rise). At the same time, the number of children under 6 years of age decreased from 1701 to 1551. This implies that the proportion of children cared for outside the home increased even more. Since day-care centre attendance represents the most prominent external risk factor for AOM, this change seems to be a good explanation for some of the increase in the occurrence of AOM.

Maternal smoking

According to the meta-analysis by Uhari et al. (1996), parental smoking is a risk factor for AOM, the relative risk being 1.7. Although male smoking has decreased in Finland, smoking among women has become more popular. Isohanni et al. (1995) reported that

smoking among pregnant women increased from 14% in 1966 to 20% in 1985-86. Changes in smoking habits may have affected the increase in the occurrence of AOM.

Increasing prevalence of allergic disorders

There is significant evidence that allergy contributes to OM as an etiologic factor (Alho et al. 1990, Juntti et al. 1999, Tikkanen et al. 2000). Especially respiratory atopy (but not atopic dermatitis) seems to be associated with RAOM (Juntti et al. 1999). Allergic rhinitis and asthma are increasing in prevalence in Western countries (Haahtela et al. 1990, Aberg et al. 1995, Linneberg et al. 1999). In Sweden, for example, the number of children with allergic disorders roughly doubled from 1979 to 1991 (Aberg et al. 1995). This alteration might be a factor associated with the increasing occurrence of AOM.

The change in the clinical picture of AOM (II)

According to the present study, the clinical picture of AOM has altered: Between the study periods the proportion of afebrile patients increased and proportion of children with spontaneous perforation of ear-drum decreased. Because of retrospective design of the study which relied on case records, these observations should be interpreted critically. Besides, it is possible that a milder clinical picture can be partly explained by an earlier consultation of a physician (Table 5). Perhaps microbiological changes (Table 4) could also play a role in the declaration of these findings, but because microbiological data from this area is lacking this causality cannot be directly inferred.

The change in the treatment of AOM (II)

A dramatic change occurred in the antibiotic treatment of AOM: in 1994-95 only 10.5% of the children were prescribed penicillin-V compared to 80.2% in 1978-79.

This cannot be explained by bacteriological changes (Nissinen et al. 1995a, Nissinen et al. 1995b). Obviously international recommendations (Bluestone and Klein 2001), intense marketing of broad spectrum drugs, and the bad taste of penicillin-V mixtures have influenced antibiotic prescription practice.

Surprisingly, in a multivariate analysis, broad-spectrum antibiotic treatment came out as a risk factor for OM recurrence. Because of the retrospective design of the study, this causality cannot be inferred. The multivariate analysis also missed important variables, like day-care attendance, and thus remained incomplete.

On the other hand, there is data from earlier studies to support this finding. In a prospective trial by Howard et al. (1976), children treated with penicillin or erythromycin suffered from fewer recurrences compared to those who received broad-spectrum drugs (13.3% vs. 40.5%, p=0.0125). Two other prospective studies failed to show differences in regard of recurrences, but they had some methodological flaws (Laxdal et al. 1970, Bass et al. 1973).

Antimicrobial agents have also been shown to induce alterations in normal nasopharyngeal flora some agents being more harmful than others. Brook and Gober (1998) compared two antimicrobial agents (amoxicillin-clavulanate and cefprozil, a second generation cephalosporin) effect on the nasopharyngeal flora in 50 children with AOM. After the completion of therapy, the cure rates were comparable and the number of potential pathogens isolated from nasopharynx were equally reduced by both therapies. However, amoxicillin-clavulanate therapy resulted in the decline of the number of interfering (protective) organisms from 50 to 11, whereas after cefprozil treatment the number was reduced only from 50 to 42 ($P< 0.001$).

Recently, significant evidence was shown on behalf of the importance of the normal flora in prevention of recurrences of AOM. A Swedish study-group used commensal α haemolytic streptococci to replace the nasopharyngeal flora in children after antibiotic treatment for AOM (Roos et al. 2001). At a three-month follow-up period in the intervention group 40% of children experienced recurrences of AOM compared to 51% of children in the control group. Moreover at three months 42% of children in the intervention group and 22% of the children in the control group had a normal tympanic

membrane. The results further suggest that antibiotic treatment itself increases the risk of recurrent OM.

In conclusion, there is more and more evidence on behalf of restoring normal, protective nasopharyngeal flora in children with AOM. So the highly recommended rationale is to use narrow spectrum antibiotics for the initial treatment of AOM. In many countries, unfortunately, the emergence of resistant bacteria, especially resistant *S. pneumoniae,* demand the use of broad-spectrum antibiotics in the treatment of AOM. On the other hand, by using a more selective therapy for AOM the proportion of resistant otitis pathogens might decrease.

The rate of OM related surgery doubled between the periods and can be explained by an increasing number of recurrent AOM attacks in the later study period. Probably there are also many other factors which may have had an impact on surgery rates like parents consciousness and attitude towards the disease and easier accessibility of an ear, nose, and throat specialist (Alho et al. 1994).

The role of cytokine genetics in the heredity of AOM (III)

At present, there is increasing evidence about the contribution of genes to the susceptibility to RAOM. Despite this, it has been difficult to discover specific genetic markers associated with the development of this disorder. Due to the multifactorial nature of AOM, it is reasonable to assume that there is no single gene responsible for RAOM. Obvious candidate genes are those controlling mucosal immunity.

Cytokines are bioactive proteins that orchestrate inflammatory and host defence responses. Constitutional polymorphism of cytokine genes may lead to individual variations in cytokine secretion (Hurme et al. 1998). Recently, cytokine genes were implicated as candidate genes in RAOM (Casselbrant and Mandel 2001). In the present study the significance of TNF-α (-308), IL-1α (-889), IL-1β (-511), IL-1β (+3953) and IL-1Ra gene polymorphism in RAOM was evaluated. The study revealed that the distribution of studied cytokine alleles did not differ significantly in the RAOM group versus the control group. However, in a subgroup of RAOM patients without a history

of allergic disorders (atopy, asthma or allergic rhinitis), allele frequencies of IL-1α (-889) differed significantly from controls (Table 7). This was mainly due to an increasing occurrence of common 1.1 genotype (OR 2.2, 95% CI 1.3-4.1).

IL-1 is a proinflammatory cytokine, found in two forms: IL-1α and IL-1β. These molecules are structurally related and share a similar profile of functions by binding to the same receptors (Dinarello 1998). They are synthesized by a variety of cell types, monocytes, macrophages, and epithelial cells for example. They act as an initiation of an inflammatory cascade by activating T cells, up-regulating the expression of adhesion molecules, and inducing the expression of other inflammatory mediators which form a cascade of inflammatory response (Dinarello 1998). The IL-1α (-889) polymorphism is located in the promonter region of IL-1 gene in chromosome 2 (See Appendix)(McDowell et al. 1995). Mutation in this region has lead to a single-base exchange (C = allele 1 to T = allele 2).

There is yet not very much data about the effect of the IL-1α alleles on the circulating IL-1α nor the clinical significance of this polymorphism. When interpreting the results of association studies it is also important to note that observed associations are not necessarily due to the effect of direct gene product on disease (van Deventer 2000). Instead genes are co-inherited with other genes, a phenomenon known as "linkage disequilibrium", and a another gene may be responsible for the phenotype.

Nevertheless, a gene supervising the production of IL-1 could be a candidate gene in RAOM because IL-1 seems to be a key mediator in the acute phase of infection of OM. In the chinchilla model of OM, IL-1β is the earliest pro-inflammatory cytokine detected in MEF (Sato et al. 1999). In AOM concentrations of IL-1β are 3 times higher in culture-positive MEF, compared with culture-negative ones (Barzilai et al. 1999). IL-1β also correlates with the age of the child: younger children have higher levels of IL-1β than older ones (Yellon et al. 1991, Juhn et al. 1992). Theoretically, not only too low but also too high cytokine production may increase the risk for AOM: If the secretion is low pathogens might not be eliminated. On the other hand, too affluent a secretion may cause the destruction of epithelium and cilia and increase the adherence of pathogens.

In general, a major problem in interpreting association studies is a high rate of false positive results, and hence the significance of these studies should be defined conservatively (van Deventer 2000). In the current work, the study group consisted of 63 individuals from 20 different families with a high occurrence of AOM. However, the control group was not ideal because the occurrence of RAOM subjects in this population was not known. Considering the multifactorial nature of the disease, the total number of RAOM subjects was rather low. Therefore, the possible association of IL-1α (-899) gene polymorphism found here needs further confirmation.

Resistance of otitis pathogens (IV)

The overall occurrence of non-susceptible *S. pneumoniae* strains in Finland is still rather low, 4-5%, compared to many other countries (Manninen et al. 1997, Kilpi et al. 2001). However, in the present study 25.0% of *S. pneumoniae* isolations were non-susceptible to penicillin. This is probably the highest figure that has ever been published in Finland. Nearly all of the *M. catarrhalis* strains produced β-lactamase. This is in concordance with other surveys from Finland (Manninen et al. 1997, Kilpi et al. 2001). Of *H. influenzae* strains 43% produced β-lactamase, which is a higher proportion than has generally been registered in Finland (Manninen et al. 1997, Kilpi et al. 2001).

The nasopharyngeal carriership of one of the three main otitis pathogens here was rather low, 29.1%, probably due to a high number of children receiving antibiotic prophylaxis at the time of surgery. On the other hand, it is in concordance with 28.3% carriership found in a recent study involving a similar population (Haddad, Jr. et al. 2000).

The high portion of non-susceptible *S. pneumonia* is an especially alarming alteration, due to the high prevalence of pneumococcal diseases and because of the difficulties in antibiotic selection in penicillin resistant cases. The risk factors for the nasopharyngeal carriage of non-susceptible *S. pneumoniae* include young age, a recent

use of antibiotics, and day-care outside home (Jackson et al. 1984, Christenson et al. 1998, Haddad, Jr. et al. 2000). Therefore, a high occurrence of penicillin resistance in the present study is probably due to the characteristics of the study population, which consisted of small children with a history of frequent antibiotic treatments.

Interestingly, all non-susceptible pneumococcal strains were isolated alone, while all pneumococcal strains isolated with ß-lactamase-producing *M. catarrhalis* were susceptible to penicillin (Table 8). This finding suggests that neighbouring ß-lactamase-producing bacteria may hinder the development of penicillin resistance of pneumococcus by reducing the selection pressure. Thus, the choice of antibiotics, which have no effect on *M. catarrhalis* in the first-line treatment of uncomplicated respiratory tract infections, could reduce the emergence of non-susceptible *S. pneumoniae*.

As shown above, it is important to note that even in Finland we have certain groups of children with a high occurrence of resistant bacteria. An optimal care of these groups plays a key role in hindering the emergence of antibiotic resistance.

Summary and conclusions

The occurrence and especially the recurrences of AOM have risen alarmingly during the past two decades. In the present study, the total rise in the number of AOM episodes was 68% between 1978-79 and 1994-95. Probably several factors contribute to this, but one of the main reasons seems to be increasing day-care attendance. Increasing prevalence of allergy, increasing maternal smoking and an increasing readiness to use of medical services may also play a role.

The clinical picture of AOM has become milder. The proportion of febrile patients has decreased, and an acute otorrhea drought spontaneously perforated ear-drum has become less common. Paradoxically, antibiotic treatment has become more aggressive. Nevertheless, more children experience sequels and need surgical treatments than 20 years ago.

The consequences of the heavy use of broad-spectrum antibiotics can be seen in an alarming emergence of resistant bacteria in risk populations. The results of the present study also suggest that a concomitant nasopharyngeal carriage of ß-lactamase-producing *M. catarrhalis* may hinder the development of the penicillin resistance of pneumococcus. Considering the low virulence of *M. catarrhalis,* the choice of antibiotics which have no effect on this pathogen in the first-line treatment of uncomplicated respiratory tract infections, could reduce the emergence of non-susceptible *S. pneumoniae*.

There is evidence that the susceptibility to RAOM is in part genetically determined. It is reasonable to assume that there is no single gene responsible for this disorder. Obvious candidate genes are, in particular, those controlling mucosal immunity. In the present study, we used a candidate gene approach to evaluate the significance of cytokine gene polymorphism in RAOM. No association was found between any studied genes (TNF-α (-308), IL-1α (-889), IL-1β (-511), IL-1β (+3953) and IL-1Ra)

and RAOM. However, in a subgroup of non-allergic individuals, the IL-1α gene allele frequencies differed significantly from those in the control group. This result should be further evaluated in a larger clinical setting with a sufficient number of subjects for a more detailed subgroup analysis.

Acknowledgments

The study was carried out at the Department of Otorhinolaryngology at the University of Tampere and Tampere University Hospital during the years 1996-2001.

First I wish to express my sincere gratitude to my supervisor Professor Juhani Pukander for introducing me to the interesting field of otitis media and epidemiology and for his support and patience through all these years.

I wish to thank my other supervisor Emeritus professor Heikki Puhakka, the former Head of the Department of Otorhinolaryngology, for giving me an opportunity to expand my view to other research fields of otitis media, for his positive attitude through these years and for his never-ending confidence in me for completing this thesis.

I am deeply grateful to Professor Mikko Hurme for his eager collaboration in the genetical part of this study.

I want to express my gratitude to Professor Pekka Laippala, who offered his expertise of statistical analysis.

I wish to thank my other co-authors, PhD Janne Aittoniemi and Docent Risto Vuento, for helping me with the microbiological questions.

I also want to thank Professor Ilmari Pyykkö, the Head of the Department of Otorhinolaryngology, for creating an inspiring atmosphere for the last months of this work.

My thanks also go to Docent Markus Rautiainen, for encouraging me to complete this work during the "low seasons" and in addition I want to thank Docent Markus Rautiainen and Mrs Anne-Mari Hakamäki for providing me an opportunity to get time off from clinical work.

My sincere thanks to my sister, MA Tuuli Jurvelin, for her fast and skillful revisions of the English language.

I am deeply grateful to the official referees, Docent Olli-Pekka Alho and Docent Markku Koskela for their careful review and excellent comments of the manuscript. I really enjoyed conversations with you.

I am also grateful to PhD Janne Hulkkonen for checking the cytokine part of this thesis.

I also want to thank PhD Hannu Raitiola and MD Jussi Karjalainen, for helping me with many practical details.

I wish to thank the personnel of the Department of Otolaryngology, for helping me with the patients in this study.

My deepest gratitude goes to my wife, Minna, for being such a good wife and mother of our children. It has been easy for me to do this work through out these years with you by my side. I also wish to thank our children, Veikka (14), Saara (11) and Laura (9), for bringing so much delight to my life.

Finally, I warmly thank the grandparents of our children, Jorma and Anja Joki-Erkkilä, and Raimo and Ritva Vasama for taking care of our children whenever was needed.

This work was supported by financial grants from the Medical Research Fund of Tampere University Hospital.

Tampere 28 November 2002

Veli-Pekka Joki-Erkkilä

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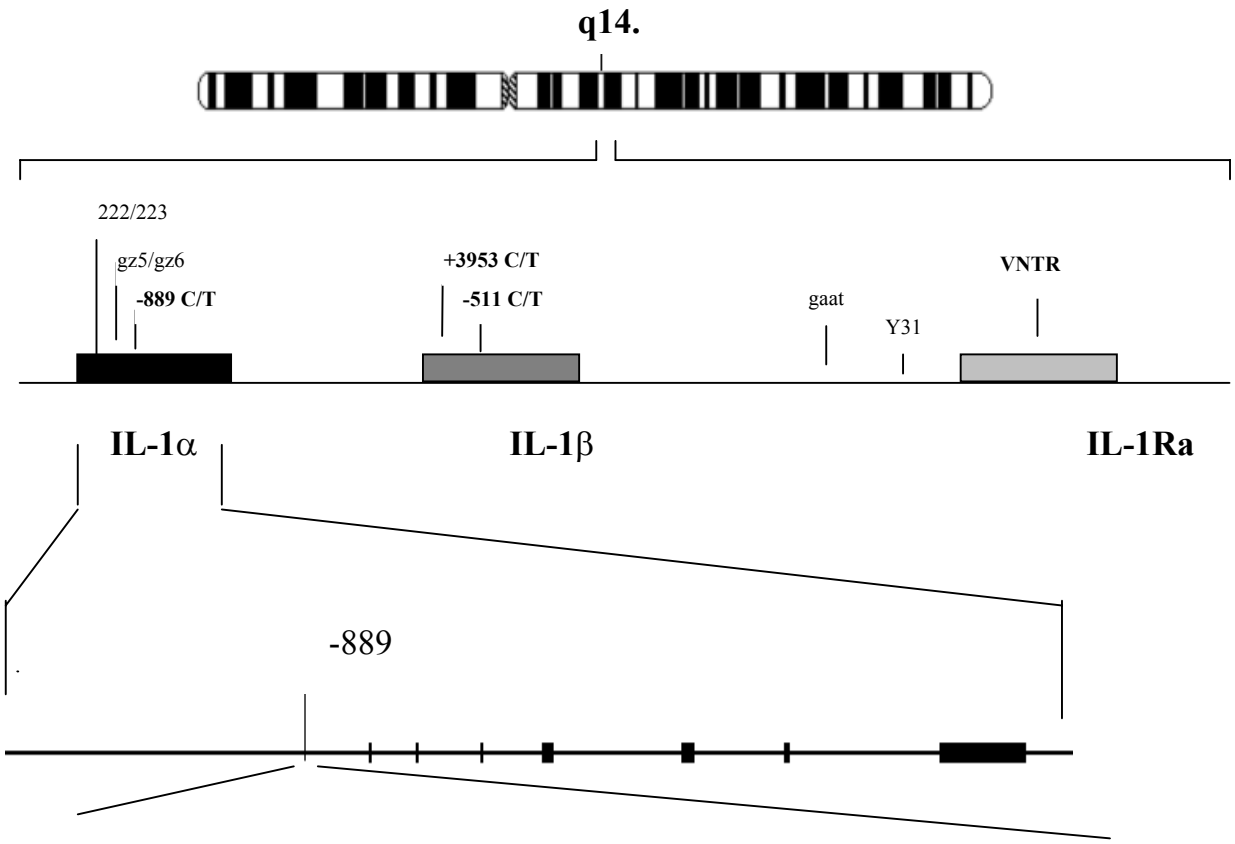
Appendix

Structure of IL-1α gene on chromosome 2 (Adapted from Hulkkonen J. Inflammatory Cytokines and Cytokine Gene Polymorphisms in Chronic Lymphocytic Leukaemia, in Primary Sjögren's Syndrome and in Healthy Subjects. Academic Dissertation. University of Tampere. Tampereen yliopistopaino Oy, Juvenes Print. Tampere. 2002)



AAGAGTCTGG**TGTTCTACCACCTGAACTAGGC**TGGCCACAGGAATTATAAAAGCTGAGAAATTCTTTAATAA
TAGTAACCAGGCAACA$^{C}/_{T}$●**CATTGAAGGCTCATATGTAA**AAATCCA