

Research

Open Access

Impact of *IL8* and *IL8-Receptor alpha* polymorphisms on the genetics of bronchial asthma and severe RSV infections

Beena Puthothu¹, Marcus Krueger¹, Jessica Heinze¹, Johannes Forster^{1,2} and Andrea Heinzmann*¹

Address: ¹University Children's Hospital, University of Freiburg, Mathildenstrasse 1, D-79106 Freiburg, Germany and ²St. Josefhospital, Sautier Str. 1, D-79104 Freiburg, Germany

Email: Beena Puthothu - beenamary@hotmail.com; Marcus Krueger - krueger@kikli.ukl.uni-freiburg.de; Jessica Heinze - jessica-heinze@web.de; Johannes Forster - Johannes.Forster@rkk-sjk.de; Andrea Heinzmann* - heinzmann@kikli.ukl.uni-freiburg.de

* Corresponding author

Published: 17 February 2006

Received: 27 December 2005

Clinical and Molecular Allergy 2006, 4:2 doi:10.1186/1476-7961-4-2

Accepted: 17 February 2006

This article is available from: <http://www.clinicalmolecularallergy.com/content/4/1/2>

© 2006 Puthothu et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: Interleukin 8 (IL8) belongs to the family of chemokines. It mediates the activation and migration of neutrophils from peripheral blood into tissue and hereby plays a pivotal role in the initiation of inflammation. Thus it is important in inflammatory lung diseases like bronchial asthma or severe infections by Respiratory Syncytial Virus (RSV). IL8 acts through binding to the IL8-Receptor alpha (IL8RA). For both genes association with asthma has been described. In addition, *IL8* has been found in association with RSV bronchiolitis. The aim of our study was to test both genes for association with asthma and severe RSV infections. In addition we were interested in whether a common genetic background of both diseases exists in regards to these genes.

Methods: We genotyped the two *IL8* promotor polymorphisms -251A/T and -781C/T and the three amino acid variants M31R, S276T and R335C in *IL8RA* on 322 children with asthma, 131 infants with severe RSV associated diseases and 270 controls. Statistical analyses made use of the Armitage's trend test for single polymorphisms and FAMHAP for calculations of haplotypes.

Results: We found association of the *IL8* polymorphism -781C/T as well as *IL8* haplotypes with asthma ($p = 0.011$ and $p = 0.036$, respectively). In addition, direct comparison of the asthmatic population with the RSV population revealed significant differences, both for -781C/T alone ($p = 0.034$) and *IL8* haplotypes ($p = 0.005$). The amino acid variants in *IL8RA* were evenly distributed in between all three populations.

Conclusion: We conclude from our data that *IL8* might play a role in the genetic predisposition to asthma and that these effects are different or even opposite to the effects on severe RSV diseases. Furthermore, *IL8RA* is unlikely to play a major role in the genetics of either disease.

Background

Interleukin 8 (IL8) is a member of the chemokine family and is produced by a wide range of cell types like monocytes, macrophages, fibroblasts and ceratinocytes. It pri-

marily mediates the activation and migration of neutrophils from peripheral blood into tissue and is involved in the initiation and amplification of inflammatory processes, which occur in the human immune system

in response to a wide variety of pathogens [1]. Thus IL8 play an important role in inflammatory lung diseases like bronchial asthma or severe infections caused by respiratory syncytial virus (RSV).

RSV, a single-stranded RNA virus, is involved in at least 70% of cases of infectious infantile bronchiolitis and has been repetitively linked to asthma. It has been hypothesized that severe RSV infection in infancy might lead to the development of recurrent wheezing and/or bronchial asthma [2,3], and consequently a common genetic background of both diseases has been discussed [4]. According to the current evidence, genetic and environmental factors determine the type of immune response to RSV infection. Furthermore, this response may affect the development of control mechanisms in the regulation of airway diseases, mainly bronchial asthma.

Increased concentrations of IL8 have been described in the bronchoalveolar fluid and sputum of asthmatic patients [5]. In addition, repeated administration of IL8 into the airways induces bronchial hyperreactivity in guinea pigs [6,7]. Genetic association of IL8 has been described with asthma [8] and RSV bronchiolitis [9,10].

IL8 binds with high affinity to two different receptors: IL8 receptor alpha (IL8RA, CXCR1) and beta (IL8RB, CXCR2). These closely related proteins are members of the superfamily of receptors, which couple to guanine nucleotide binding proteins. *IL8RA* is localized on chromosome 2q35 [11], where linkage to total serum IgE levels in asthmatics has been described [12]. In addition, association of *IL8RA* polymorphisms has recently been described with asthma and chronic obstructive pulmonary disease [13].

We were interested in the relationship between severe RSV infection and/or bronchial asthma and *IL8* and *IL8RA* polymorphisms in the German population. We chose to study two *IL8* promoter polymorphisms (-251A/T and -781C/T) and three amino acid variants in *IL8RA* (M31R, S276T and R335C), which are located in the N-terminus of the protein, in the third extracellular loop and in the C-terminus of the protein respectively. The selection of the *IL8* polymorphisms was based on previous studies in our

asthmatic and control populations, in which these polymorphisms were shown to be most informative [8].

Materials and methods

Subjects

Asthmatic population

322 children with bronchial asthma (aged 5 to 18 years) were recruited from the South-Western part of Germany between July 2000 and January 2005. All probands were characterized at the University Children's Hospital, Freiburg, Germany using a standardized clinical protocol. An extended medical history was recorded including the occurrence and duration of wheezing symptoms; previous and acute medications; severity of previous asthma attacks; previous allergic rhinitis or conjunctivitis; atopic dermatitis and any family history of allergic diseases.

The diagnosis was based on a clear-cut history of asthmatic symptoms; the use of anti-asthmatic medication and the presence of bronchial hyper-reactivity. The anti-asthmatic drugs included typical betamimetics like salbutamol and standard corticosteroids used in asthma treatment like budesonide. Bronchial hyper-reactivity was defined as a fall in forced respiratory volume in one second (FEV1) by at least 15% in histamine testing or exercise provocation, using standard protocols [14]. The exact recruitment procedure has been described in detail previously [15].

Population of children with severe RSV infection

This population was recruited at the University Children's Hospital, Freiburg, Germany and also in the Community Children's Hospital, St. Josef's Hospital, Freiburg. Infants and children were eligible when hospitalized due to RSV infection between September 1998 and March 2005 at an age of less than 2 years. RSV infection was detected by antigen test and/or RSV-specific PCR [16]. According to the case definition, children had symptoms of bronchiolitis, for example wheezing and tachypnoe and needed either supplementary oxygen and/or gavage feeding and/or intravenous fluids. Children with congenital heart defects, immune deficiency or chromosomal aberrations were excluded. DNA samples were collected either by

Table 1: Frequency and HWE of the polymorphisms within the three populations.

Polymorphism	Asthma Frequency	HWE	RSV Frequency	HWE	Controls Frequency	HWE
<i>IL8</i> rs4073 (-251A/T)	0.441	0.469	0.470	0.241	0.493	0.223
<i>IL8</i> rs2227306 (-781C/T)	0.618	0.708	0.540	0.914	0.543	0.242
<i>IL8RA</i> rs16858811 (M31R)	0.969	1.000	0.974	1.000	0.954	1.000
<i>IL8RA</i> rs16858809 (S276T)	0.933	1.000	0.948	1.000	0.944	1.000
<i>IL8RA</i> rs16858808 (R335C)	0.967	1.000	0.974	1.000	0.954	0.441

Results of genotyping of the *IL8* and *IL8RA* variants on the three populations. The frequency is given for the wildtype allele. Also given is the p-value for the Hardy Weinberg Equilibrium (HWE) as calculated by Finetti.

Table 2: Genotype distribution and p-values for association

Polymorphism	Genotype distribution			p-values for association		
	Asthma	RSV	Controls	Asthma- Controls	RSV- Controls	Asthma- RSV
<i>IL8</i> rs4073 (-251A/T)	64; 148; 101	26; 73; 34	70; 124; 74	0.087	0.550	0.425
<i>IL8</i> rs2227306 (-781C/T)	123; 147; 48	37; 62; 27	84; 124; 61	0.011	0.937	0.034
<i>IL8RA</i> rs16858811 (M31R)	299; 20; 0	128; 7; 0	245; 25; 0	0.174	0.152	0.655
<i>IL8RA</i> rs16858809 (S276T)	239; 30; 0	121; 14; 0	121; 14; 0	0.409	0.812	0.376
<i>IL8RA</i> rs16858808 (R335C)	298; 21; 0	127; 7; 0	246; 23; 1	0.239	0.171	0.584

The genotype distribution within the three different populations is given in the following order: homozygous wildtype, heterozygous and homozygous mutation. Also listed are the p-values for association with the diseases as calculated by the Armitage's trend test.

blood taking or buccal smears with sterile cotton sticks. In total 131 children were included in this study.

Control population

270 randomly chosen probands, aged 19 to 40 years, served as controls. They originated from the same area in the South-Western part of Germany. No medical history was taken and no medical testing was performed on controls.

Genotyping

DNA was extracted from peripheral blood leucocytes or buccal smears following standard protocols and column purified (DNA midikit, Qiagen, Germany). The two *IL8* polymorphisms (-251A/T and -781C/T) were typed as described previously [8].

The three *IL8R* polymorphisms were typed by means of restriction fragment length polymorphism (RFLP):

Met31Arg (rs16858811) was typed using the primer pair 5'-TGAAGATTACAGGCCCTGTA-3' and 5'-AAATC-CAGCCATTACCTTG-3'. Following PCR, the product was digested with two units of BglI (Fermentas) at 37° over night and the fragments were resolved on a 4% agarose gel.

Ser276Thr (rs16858809) was typed using the primer pair 5'-TCACCCTGCGTACACTGTTT-3' and 5'-GCCAA-GAACTCCTTGCTGAC-3. Following PCR, the product was digested with one unit of Alw261 (Fermentas) at 37°C over night and the fragments were resolved on a 4% agarose gel.

Arg335Cys (rs16858808) was typed using the primer pair 5'-AGGAGTTCTTGGCACGTGAT-3' and 5'-AATGATGGT-GCTTCGTTTCC-3'. Following PCR, the product was digested with one unit of DpnII (NEB) at 37°C over night and the fragments were resolved on 4% agarose gel.

Sequencing

For each polymorphism, three controls (homozygous wild type, heterozygous and homozygous mutation) were

sequenced by the dideoxy chain termination method [17], using the Big Dye Terminator cycle sequencing kit on an ABI 310 sequencer (Applied Biosystems). All the following studies included these reference individuals.

Statistical analysis

Association analysis was performed for each polymorphism using Armitage's Trend Test. This test takes into account the individuals' genotypes rather than just the alleles, following the guidelines given by Sasieni [18], as implemented in the program Finetti (Thomas F. Wienker, unpublished data; <http://ihg.gsf.de/cgi-bin/hw/hwa1.pl> and <http://ihg.gsf.de/linkage/download/finetti.zip>). Hardy Weinberg equilibrium (HWE) was calculated for each polymorphism in all three populations using also the program Finetti. In addition to analyses based on single polymorphisms, we performed haplotype frequency estimations using the program FAMHAP [19]. The extent of linkage disequilibrium between polymorphisms has been calculated by Arlequin.

Approval

The collection of blood and the experimental procedures were approved by the Ethical Committee of the University of Freiburg. A statement of informed consent was signed by all participants; or in the case of children, signed by their parents.

Results

Genotyping

Five polymorphisms, two *IL8* and three *IL8RA* polymorphisms, were typed on 131 children with severe RSV infection, 270 controls and 322 asthmatic children. The allelic frequencies of the polymorphisms in the three populations and the Hardy Weinberg equilibrium are given in table 1. All polymorphisms were in Hardy Weinberg equilibrium in all populations.

Association studies

The genotype distribution in the populations and the p-values for association, obtained by Armitage's Trend Test, are listed in table 2. An association was observed between asthma and the promoter polymorphism -781C/T in *IL8*

Table 3: Frequency of the haplotype within the three populations.

	Haplotype	Asthma	RSV	Controls
IL8	1 1	0.061	0.029	0.038
	1 2	0.380	0.443	0.455
	2 1	0.558	0.503	0.504
	2 2	0.002	0.024	0.004
IL8RA	1 1 1	0.904	0.922	0.897
	1 2 1	0.065	0.052	0.055
	2 1 2	0.031	0.026	0.044

Number 1 refers to the wildtype allele, 2 to the variant allele.

($p = 0.011$). When comparing children with asthma to children with severe RSV infection, a significant different allelic distribution was observed for the same polymorphism ($p = 0.0342$). The other evaluated polymorphisms showed no association with bronchial asthma or severe RSV infection.

The two polymorphisms within IL8 were in very tight linkage disequilibrium in all populations ($p = 0.00000$ for all possible pairs). Thus we did not perform Bonferroni correction for multiple testing as it would be far too conservative in this case. In IL8RA M31R and R35C were transmitted together ($p < 0.0001$ for all populations).

Haplotype analyses

The haplotype pattern in all populations is given in table 3. IL8 haplotypes showed weak association with asthma ($p = 0.036$). Furthermore, haplotypes of IL8 were significantly differently distributed between asthmatics and children with severe RSV infection ($p = 0.005$; see table 4). In contrast, no effect was seen with IL8RA haplotypes on either disease.

Discussion

So far, only few studies have investigated the impact of polymorphisms within the IL8 signaling pathway on the genetic background of severe RSV associated diseases or bronchial asthma. The IL8 pathway includes IL8 itself, its two receptor chains IL8RA and IL8RB and its degradative enzyme aminopeptidase N [20]. In a previous study, we have shown that IL8 polymorphisms are in association with bronchial asthma in the German population using 230 asthmatic children and 270 randomly chosen controls [8]. Furthermore, we hypothesized that polymorphisms within IL8 have opposite effects on the development of asthma and severe RSV infections. The hypothesis was based on the observation that the -251T allele was more common in patients with asthma than in controls. In contrast, two studies from Hull and colleagues have demonstrated that the opposite allele, that is

Table 4: Results of the haplotype analyses using FAMHAP.

	Asthma-Controls	RSV-Controls	RSV-Asthma
IL8	0.036	0.180	0.005
IL8RA	0.448	0.729	0.724

-251A, is associated with severe RSV infection in the English population [9,10].

Thus the first aim of the current study was to test whether our initial hypothesis of an opposite role of IL8 polymorphisms on asthma and RSV infections holds in a population of German children with severe RSV associated diseases. Second, we wanted to verify the association between bronchial asthma and IL8 polymorphisms in an extended asthmatic population (increased by 90 asthmatic children to 320 probands). Thirdly, we extended our investigation to include IL8RA as recently association of asthma and chronic obstructive pulmonary disease with this gene has been reported in another German samples [13].

In the here presented study, the polymorphisms -251A/T and -781C/T within IL8 showed no association with severe RSV associated diseases - neither in analyses of single polymorphisms nor in haplotype analyses. This might suggest that IL8 does not play a major role in the development of severe RSV infections in the German population. These findings are in contrast to the above mentioned studies of Hull *et al.* [9,10]. The discrepancy could be due to several reasons: The infants included in the study of Hull *et al.* were younger than in our study. Furthermore, the inclusion criteria differed slightly between both studies, for example, Hull and colleagues included children with pre-existing heart diseases, whereas we excluded those children. Finally, though the English and German population represent both Caucasians it is well known that the genetic predisposition to allergic diseases are at least partially different between Germans and English people [21].

The association between bronchial asthma and -781C/T within IL8 could be confirmed in an extended asthmatic population ($p = 0.011$), whereas the association with the second IL8 polymorphism -251A/T and asthma became weaker and was no longer statistically significant ($p = 0.087$). This might be in accordance to one study, showing that -251A/T has no functional impact, whereas the base pair substitution C to T at position -781 within the IL8 promotor enhances the binding of transcription factors and thus is probably more important in the genetic regulation of IL8 expression [22].

Furthermore, a direct comparison of the asthmatic population with the RSV population revealed significant differences, both for -781C/T alone ($p = 0.034$) as well as in the haplotype analyses ($p = 0.005$). These results may support our initial hypothesis, that polymorphisms within *IL8* have opposite effects in the pathophysiology of asthma and RSV associated diseases. However, one should notice, that the allelic frequencies of the polymorphisms did not markedly differ between controls and children with severe RSV infections. Thus the positive association might merely reflect the fact that the genotype frequencies in asthmatics differ from controls.

In contrast to Stemmler *et al.*, who found that the *IL8RA* polymorphisms M13R and R335C were significantly associated with bronchial asthma [13], we found no association of these polymorphisms neither with asthma nor severe RSV associated diseases. Again the conflicting results might be due to different inclusion criteria. The study by Stemmler *et al.* used 68 adult patients and 130 children with asthma whereas we used exclusively children with asthma. The "adult phenotype" of asthma is quite different from the asthmatic phenotype in children. For example pediatric asthma is much more often allergic than asthma in adults. Thus it might not be surprising that the genetic background of asthma differs between adults and children [23]. Furthermore, the size of our asthmatic study population was larger than the population of Stemmler *et al.* (198 asthmatic patients versus 320 patients). Thus the significant result in their study might just reflect a type 1 error. However, as the risk factor conferred by a single gene is quite small in complex genetic diseases like asthma, it is also possible that we missed true association in our population due to a type 2 error.

Conclusion

We conclude from our data, that *IL8* might play a role in the genetic predisposition to bronchial asthma and that these effects are different, or maybe even opposite to the effects of the same polymorphisms on severe RSV associated diseases. In contrast *IL8RA* polymorphisms do not play a major role, neither in the development of severe RSV infections nor in asthma.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

BP performed genotyping and sequencing of *IL8* polymorphisms as well as the statistical analyses and drafted the manuscript.

MK participated in the clinical design of the study and the recruitment of the RSV population.

JH performed genotyping and sequencing of *IL8RA* polymorphisms

JF participated in the clinical design of the study.

AH conceived and coordinated the study and helped to draft the manuscript.

All authors have read and approved the final manuscript.

Acknowledgements

This project was supported by grants from the Deutsche Forschungsgemeinschaft (DFG HE 3123/3-1 and DFG HE 3123/4-2). The recruitment of asthmatic children was partially supported by a grant from the NIH (NIH R01 HL66533-01).

References

1. Harada A, Sekido N, Akahoshi T, Wada T, Mukaida N, Matsushima K: **Essential involvement of interleukin-8 (IL8) in acute inflammation.** *J Leukoc Biol* 1994, **56**:559-564.
2. Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson F, Kjellman B: **Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13.** *Am J Respir Crit Care Med* 2005, **171**:137-41.
3. Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B: **Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7.** *Am J Respir Crit Care Med* 2000, **161**:1501-7.
4. Stein RT, Sherrill D, Morgan W, Holberg CJ, Halonen M, Taussig LM, Wright AL, Martinez FD: **Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years.** *Lancet* 1999, **354**:541-5.
5. Norzila MZ, Fakes K, Henry RL, Simpson J, Gibson PG: **Interleukin-8 secretion and neutrophil recruitment accompanies induced sputum eosinophil activation in children with acute asthma.** *Am J Respir Crit Care Med* 2000, **161**:769-74.
6. Fujimura M, Xiu Q, Tsujimura M, Tachibana H, Myou S, Matsuda T, Matsushima K: **Role of leukotriene B4 in bronchial hyperresponsiveness induced by interleukin-8.** *Eur Respir J* 1998, **11**:306-11.
7. Xiu Q, Fujimura M, Nomura M, Saito M, Matsuda T, Akao N, Kondo K, Matsushima K: **Bronchial hyperresponsiveness and airway neutrophil accumulation induced by interleukin-8 and the effect of the thromboxane A2 antagonist S-1452 in guinea-pigs.** *Clin Exp Allergy* 1995, **25**:51-9.
8. Heinzmann A, Ahlert I, Kurz T, Berner R, Deichmann KA: **Association study suggests opposite effects of polymorphisms within IL8 on bronchial asthma and respiratory syncytial virus bronchiolitis.** *J Allergy Clin Immunol* 2004, **114**:671-6.
9. Hull J, Ackerman H, Isles K, Usen S, Pinder M, Thomson A, Kwiatkowski D: **Unusual haplotypic structure of IL8, a susceptibility locus for a common respiratory virus.** *Am J Hum Genet* 2001, **69**:413-9.
10. Hull J, Thomson A, Kwiatkowski D: **Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families.** *Thorax* 2000, **55**:1023-7.
11. Holmes WE, Lee J, Kuang WJ, Rice GC, Wood WI: **Structure and functional expression of a human interleukin-8 receptor.** *Science* 1991, **253**:1278-80.
12. Xu J, Postma DS, Howard TD, Koppelman GH, Zheng SL, Stine OC, Bleeker ER, Meyers DA: **Major genes regulating total serum immunoglobulin E levels in families with asthma.** *Am J Hum Genet* 2000, **67**:1163-73.
13. Stemmler S, Arinir U, Klein W, Rohde G, Hoffjan S, Wirkus N, Reinitz-Rademacher K, Bufe A, Schultze-Werninghaus G, Epplen JT: **Association of interleukin-8 receptor alpha polymorphisms with chronic obstructive pulmonary disease and asthma.** *Genes Immun* 2005, **6**:225-30.
14. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee: **Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC.** *Lancet* 1998, **351**:1225-32.

15. Heinzmann A, Jerkic SP, Ganter K, Kurz T, Blattmann S, Schuchmann L, Gerhold K, Berner R, Deichmann KA: **Association study of the variant Arg110Gln in Interleukin-13 with atopic diseases and juvenile idiopathic arthritis.** *J Allergy Clin Immunol* 2003, **112**:735-39.
16. Forster J, Ihorst G, Rieger CH, Stephan V, Frank HD, Gurth H, Berner R, Rohwedder A, Werchau H, Schumacher M, Tsai T, Petersen G: **Prospective population-based study of viral lower respiratory tract infections in children under 3 years of age (the PRI.DE study).** *Eur J Pediatr* 2004, **163**:709-16.
17. Sanger F, Nicklen S, Coulson AR: **DNA sequencing with chain-terminating inhibitors.** *Biotechnology* 1992, **24**:104-8.
18. Sasieni PD: **From genotypes to genes: doubling the sample size.** *Biometrics* 1997, **53**:1253-61.
19. Becker T, Knapp M: **Maximum-likelihood estimation of haplotype frequencies in nuclear families.** *Genet Epidemiol* 2004, **27**:21-32.
20. Palter SF, Mulayim N, Senturk L, Arici A: **Interleukin-8 in the human.** *J Clin Endocrinol Metab* 2001, **86**:2660-7.
21. Kurz T, Strauch K, Heinzmann A, Braun S, Jung M, Ruschendorf F, Moffatt MF, Cookson WO, Inacio F, Ruffilli A, Nordskov-Hansen G, Peltre G, Forster J, Kuehr J, Reis A, Wienker TF, Deichmann KA: **A European study on the genetics of mite sensitization.** *J Allergy Clin Immunol* 2000, **106**:925-32.
22. Hacking D, Knight JC, Rockett K, Brown H, Frampton J, Kwiatkowski DP, Hull J, Udalova IA: **Increased in vivo transcription of an IL8 haplotype associated with respiratory syncytial virus disease-susceptibility.** *Genes Immun* 2004, **5**:274-82.
23. Bottema RW, Reijmerink NE, Koppelman GH, Kerckhof M, Postma DS: **Phenotype definition, age, and gender in the genetics of asthma and atopy.** *Immunol Allergy Clin North Am* 2005, **25**:621-39.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

