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Familial aggregation of tinnitus: a European multicentre study

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Abstract. *Introduction and aim*: Tinnitus is a common condition affecting approximately 20% of the older population. There is increasing evidence that changes in the central auditory system following cochlear malfunctioning are responsible for tinnitus. To date, few investigators have studied the influence of genetic factors on tinnitus. The present report investigates the presence of a familial effect in tinnitus subjects.
Methods: In a European multicentre study, 198 families were recruited in seven European countries. Each family had at least 3 siblings. Subjects were screened for causes of hearing loss other than presbyacusis by clinical examination and a questionnaire. The presence of tinnitus was evaluated with the question “Nowadays, do you ever get noises in your head or ear (tinnitus) which usually last longer than five minutes”. Familial aggregation was tested using three methods: a mixed model approach, calculating familial correlations, and estimating the risk of a subject having tinnitus if the disorder is present in another family member.
Results: All methods demonstrated a significant familial effect for tinnitus. The effect persisted after correction for the effect of other risk factors such as hearing loss, gender and age. The size of the familial effect is smaller than that for age-related hearing impairment, with a familial correlation of 0.15.
Conclusion: The presence of a familial effect for tinnitus opens the door to specific studies that can determine whether this effect is due to a shared familial environment or the involvement of genetic factors. Subsequent association studies may result in the identification of the factors responsible. In addition, more emphasis should be placed on the effect of role models in the treatment of tinnitus.

Introduction

Tinnitus is defined as an auditory perception in the absence of an external source of sound.[1] Several studies have shown a high prevalence of tinnitus in ageing populations. Recently, the Blue Mountain hearing study[2] reported that, in an epidemiological study of a population of subjects aged 55 years and older, 30% reported the presence of tinnitus. These findings are in line with previous papers, which reported a prevalence of approximately 30%.[3,4] As the average age of our society continues to increase, the elucidation of the pathophysiology of tinnitus will gain in importance.

Several factors are known to influence tinnitus. Hearing loss[3,5-8] and noise exposure[3,8,9] are the best documented. However, several other risk factors such as increasing age,[10] drug-induced ototoxicity,[11] dietary factors,[12,13] alcohol consumption, hypertension, elevated blood lipids, liver disease, cervical arthritis and socio-economic factors[14] have been suggested. Neurophysiological investigations have explored the central nervous system to decipher the pathophysiology of tinnitus. This resulted in the development of the hypothesis that changes in the central auditory system following dysfunction of the cochlear

receptors are responsible for tinnitus.[1]

The possibility of genetic factors being involved in the development of tinnitus has only been explored to a limited extent. Tyler *et al.*[15] recently stated that there might be a genetic factor associated with tinnitus.[15] However, little research has been undertaken since to confirm this hypothesis in humans. Several known monogenic conditions causing hearing loss are associated with tinnitus, e.g. WFS1[16] and COCH.[17] Furthermore, genetic factors may play a role in the dysfunction of the central auditory pathway.[15]

The presence of genetic factors for a certain trait can be studied by analysing familial aggregation, which implies that a trait is more commonly found among the family members of an affected person than in the general population. This may be linked to deficiencies in certain genes, but also to a shared familial environment, or possibly a combination of both factors. If familial aggregation is present, the second step will consist of estimating the proportion of phenotypic variance as a result of genetic differences, a feature referred to as "heritability".

This study reports on familial aggregation in subjects recruited by seven European centres. We calculate the relative risk of tinnitus for subjects who have a sibling with tinnitus. As far as we know, this is the first study of familial aggregation or of tinnitus heritability.

Materials and methods

Setting

The present study is part of a larger European study on Age-Related Hearing Impairment (ARHI). This project aims to identify environmental and genetic factors for age-related hearing impairment. To this end, a family-based study was designed in which seven research groups from six European countries recruited subjects.

Study Design

In order to study the familial aggregation of tinnitus we investigated the presence of tinnitus in siblings of large families, with a minimum of three siblings per family. Large families were recruited in order to maximise the accuracy of the estimated effects. All subjects were asked the same question: "Nowadays, do you ever get noises in your head or ear (tinnitus) which usually last longer than five minutes". The possible answers were "yes", "no" or "missing". Depending on their response, the subjects were categorised as experiencing tinnitus or not. We used two approaches to assess the familial aggregation of tinnitus: the first approach involved all subjects with the aim of identifying a possible familial effect linked to tinnitus, while the second approach focused on the first person recruited (i.e. the proband) in each family and investigated whether the presence of tinnitus in siblings increased the probability of tinnitus in this individual. Using the information about the siblings in each family, subjects were categorised into those having at least one affected sibling and those having no affected siblings.

Study subjects

Seven centres from six European countries recruited Caucasian volunteers between 50 and 75 years of age using population registers, or through clinical consultation at the respective Ear, Nose and Throat (ENT) and Audiological Medicine departments. An otologically normal population was identified with families comprising at least three siblings. An extended questionnaire relating to medical history and exposure to environmental factors such as noise and solvents was completed by all volunteers. Strict exclusion criteria based on general medical conditions were applied. A full list can be found in Appendix 1. All subjects with general medical conditions that may have affected hearing thresholds were excluded from this study. Exposure to noise or solvents, however, was not regarded as an exclusion factor.

All subjects underwent otoscopic investigation by an ENT doctor/audiological physician to exclude the presence of a middle ear problem. Hearing thresholds were measured in a sound-treated booth. A modified Hughson-Westlake method was used to test air conduction thresholds at 0.25, 0.5, 1, 2, 4, and 8 kHz, and bone conduction at 0.5, 1, 2, and 4 kHz, for all participating subjects. The audiological exclusion criteria were: a conductive hearing loss of more than 15 dB averaged over 0.5, 1 and 2 kHz or an asymmetrical hearing loss with a difference in air conduction thresholds exceeding 20 dB in at least 2 frequencies between 0.5, 1 and 2 kHz. The Pure Tone Average (PTA) using four frequencies (0.5, 1, 2, 4 kHz) was calculated. Written informed consent was obtained from all participants prior to any form of investigation. The ethics committees of all the participating centres approved the study.

Statistical analysis

Chi-square testing was used to validate a possible difference in prevalence between males and females. Differences in age and PTA between the group with and without tinnitus were tested with an unpaired T-test and a Kruskal-Wallis test respectively.

Generalised linear mixed model (GLMM) analysis

Three statistical methods were applied to identify possible familial effects in tinnitus. First of all, a generalised linear mixed model (GLMM) analysis was performed. The binary outcome variable was the presence of tinnitus. A logistic model was assumed with standardised age, sex and standardised PTA (pure tone average of 0.5, 1, 2 and 4 kHz), and recruitment centre as covariates. Testing also took place to determine whether there was a significant interaction between sex and the other covariates. Age and PTA were standardised for computational reasons by subtracting the mean for each subject and dividing it by the standard deviation. Family was considered to be a random effect, i.e. the association between family members was modelled through the inclusion of random intercepts. A random intercept variance that significantly differs from zero provides evidence for familial clustering. Models with and without random effects were therefore compared using likelihood ratio (LR) tests and by comparing the Akaike Information Criteria (AIC) values. LR testing was carried out using non-standard LR tests since the null hypothesis is on the boundary of the parameter space (see, for example, Molenberghs and Verbeke[18]).

To integrate the integrals numerically in the marginal likelihood, adaptive Gaussian quadrature was used. Newton-Raphson iteration was used to maximise the log likelihood. We varied the number of quadrature points to see whether the approximation to the marginal likelihood could be improved further. Convergence of the models was carefully checked by inspecting the gradient values and the final Hessian matrix.

Familial CORrelations (FCOR)

We calculated the familial correlation using the FCOR (Familial CORrelations) program of the Statistical Analysis in the Genetic Epidemiology (SAGE) software package.[19] This method estimates multivariate familial correlations, and their asymptotic standard errors for different relationship types, in our case sister-sister, brother-sister, brother-brother and sibling-sibling. Using the corresponding sample size and standard error, we used the Fisher Z-transformation to test for significance. To test for differences between the relationship types, we used the homozygosity test in the FCOR programme, which is an asymptotic test for determining if the correlation matrices for the subtypes (e.g. sister-sister) of a given main type (e.g. sibling-sibling) correlation are equal.[20]

Relative risk (RR) estimation

In order to estimate the impact of the familial aggregation, we investigated whether the presence of a family member with tinnitus influences the risk of tinnitus in siblings. To avoid possible issues relating to subject dependency, we used only the probands for these analyses. Logistic regression is suitable for identifying factors associated with a binary outcome variable. However, if the outcome variable has a high prevalence, the odds ratio obtained by the logistic regression tends to overestimate the relative risk when >1 and to underestimate the relative risk when <1.[21] In our study group, the prevalence rate for tinnitus was 21%, so a logistic regression model was not considered appropriate. We therefore used a Cox proportional hazard model, assuming a constant risk period. This model estimates conditional hazard ratios which were adapted to estimate Relative Risk (RR) for cross-sectional data.[22] The risk factors included in the analysis were age, gender, duration of noise exposure, migraine, arthritis, hearing loss and the presence of a sibling with tinnitus. These analyses were performed using the GENMOD, NLMIXED and PHREG procedure of SAS, Version 9.1.3 of the SAS System for Windows.

Results

Descriptions of the recruited families

A total of 198 families were recruited. The number of siblings ranged from 3 to 12, with a median of 5. Overall, 981 subjects were included. Table 1 shows an overview of the families recruited by each centre.

Gender and hearing threshold differences across the entire study population

From the entire study population, 208 subjects answered “yes” to the question of whether they experienced tinnitus. This resulted in a

Table 1
Number of subjects and families per recruitment centre

Recruitment Centre	Number of subjects	Number of Families	Number of siblings			Number of subjects with tinnitus	Percentage of subjects with tinnitus
			Median	Minimal	Maximal		
Antwerp	330	65	5	3	9	60	18%
Copenhagen	119	27	4	3	6	23	19%
Tuebingen	68	15	5	3	6	26	38%
Oulo	60	12	5	4	7	17	28%
Tampere	146	32	5	4	6	35	16%
Padova	78	16	5	3	7	17	21%
Nijmegen	180	31	6	3	12	30	17%
Total	**981**	**198**	**5**	**4**	**5**	**208**	**21%**

prevalence of 21.2%. Of the male participants, 25.2% (113 out of 449) experienced tinnitus; the figure for the female participants was 17.8% (95 out of 532). Chi-square testing showed that this difference was significant ($P < 0.01$). The average age of a subject experiencing tinnitus was 60.9 (Standard Deviation = 6.3) years of age, while in the group without tinnitus the average age was 61.4 years of age (SD = 6.1), both with a normal distribution (data not shown). A standard t-test for unpaired measures revealed no significant difference between the two groups. The mean PTA for the group with tinnitus was 23.0 dB (SD = 11.8) and 19.3 dB for the group without tinnitus (SD = 10.6), both with a non-normal distribution (data not shown). The Kruskal-Wallis test indicated that this difference between both groups was highly significant ($P < 0.0001$).

Gender and hearing threshold differences in the probands only

Of the 198 probands, 43 experienced tinnitus, a prevalence rate of 21.7%. Of the male participants, 28.9% (24 out of 83) experienced tinnitus; the figure for the female participants was 16.5% (19 out of 115) (chi-square test, p-value <0.05). The mean age of the subjects with tinnitus was 61.0 years of age (SD = 5.5), as opposed to 61.3 years of age (SD = 5.2) for the subjects without tinnitus. This difference was not significant. The average PTA of subjects with tinnitus was 22.1 dB (SD = 12.3), while subjects without tinnitus had an average PTA of 20.3 dB (SD = 11.8). Again, this difference between the groups was not significant (Kruskal-Wallis test).

The mixed model applied to the entire study population

Table 2 shows the results for the mixed model analysis. The mixed model analysis determines the contribution of the following factors to the presence of tinnitus: standardised age, gender, standardised PTA and the familial identifier (the family to which each subject belongs). The effect of each factor is represented by the estimate, while the standard error and p-value indicate significance. To assess the proportion of the familial effect, the family identifier was modelled as a random effect. This method made it difficult to interpret the extent of the familial effect. However, it allowed for the correction of confounding factors. The presence of tinnitus was found to be familially aggregated and this remained significant after correction for standardised age, gender and standardised PTA.

Results of the familial correlation in the whole population

Using the FCOR programme in SAGE we calculated familial correlations for siblings with and without tinnitus. It is important to note that, with this technique, we cannot take confounding factors such as age, sex and hearing level into consideration. The sibling-sibling correlation is 0.16 with a standard error of 0.03 (95% confidence interval 0.25 to 0.06, p-value 0.001). Homogeneity testing of the correlations between sister-sister, brother-brother and sister-brother showed no significant difference (chi-square 0.66, p-value 0.72).

Table 2
Results of the mixed model analysis

Dependent variable: Tinnitus **Independent variables**	**All subjects** **Estimate ± SE**	**p**
Fixed effects	-2.2412 ± 0.3458	<0.001
Standardised Age	-0.1798 ± 0.1018	0.08
Gender	0.5626 ± 0.1901	<0.05
Standardised PTA	0.4409 ± 0.1024	<0.001
Random effect		
Family identifier	1.1189 ± 0.1685	<0.001

The dependent variable is tinnitus. Family identifier is considered as a random effect, and standardised age, standardised PTA and Gender as fixed effects.

Table 3
Results for the relative risk estimate

	Cox Proportional Hazard Model		
Covariate	**Hazard Ratio**	**95% CI**	**P-value**
Age	0.9968	0.97-1.05	0.845
Gender	1.1081	0.83-1.48	0.481
PTA	1.0000	0.98-1.01	0.992
Having a family member with tinnitus	1.7045	1.21-2.40	<0.005

Tinnitus was a dependent variable. Age, Gender, PTA and Having a family member with tinnitus were dependent variables.

Relative risk estimation: does a family member with tinnitus increase the probability of tinnitus for the proband?

The risk factors of age, gender, duration of noise exposure, migraine, arthritis, family member with tinnitus and PTA were individually tested in the Cox proportional hazard model (data not shown). Only the effect of a family member with tinnitus was significant. The results of the mixed model analyses showed effects for PTA, gender and age. This is consistent with the literature and so we included PTA, gender and age in our final analysis. The final model was constructed using the aforementioned factors as well as the variable 'having a family member with tinnitus' (Table 3). These results show that subjects related to a sibling with tinnitus were 1.7 times more likely to have tinnitus themselves than subjects from a family without tinnitus.

Discussion

The main finding of this multicentre family study was that, in 981 otologically normal subjects from 198 European families, the presence of tinnitus had a familial component. The study population used in this study represented an ideal otological population, as all subjects experienced hearing loss as a result of presbyacusis or the influence of environmental factors, and not because of any otological conditions. Furthermore, no subjects were suffering from disorders with a potential affect on hearing, as summarised in the appendix. The mixed model analysis showed that this finding was independent of differences in age, gender and hearing thresholds. As a result it can be stated that the perception of tinnitus was influenced by familial factors independent of other patient characteristics. By applying familial correlations we were able to estimate the correlation between siblings and to compare these figures with previously published figures for ARHI. However, the data we obtained should be treated with caution because the familial correlation analysis could not account for possible confounding factors. Using a Cox-proportional model we estimated that tinnitus in a sibling raises the probability of tinnitus by a factor of 1.7. This effect persisted even after correction for several known risk factors such as age, gender, hearing loss, migraine, arthritis and noise exposure.

In our study population, the prevalence of tinnitus was 21.2%. In the Blue Mountains Hearing Study (BMHS), Sindhusake *et al*.[23] reported a prevalence of 30.3% in a population aged above 55 years, which is in line with other reports.[3,4] The BMHS included the following question: "Have you experienced any prolonged ringing, buzzing or other sounds in your ears or head within the past year … that is lasting for five minutes or longer?". We asked a similar question, although the wording was slightly different. Small differences in tinnitus definition have been found to result in different tinnitus prevalence rates.[24] In our study, then, the difference in prevalence could be explained by a difference in the definition of tinnitus. However, a more likely explanation is that we excluded subjects with otological disease, and subjects from this category were included in the BMHS.

Another obvious finding from our study is that tinnitus is more prevalent among males (21.2%) than females (17.8%). In addition, we observed a significant correlation between PTA hearing loss and the presence of tinnitus. Other investigators have also identified hearing loss[3,5-8] and sex[2] as risk factors for developing tinnitus. We were unable to confirm other previously published risk factors such as age,[10] drugs-induced ototoxicity,[11] dietary factors,[12,13] alcohol consumption, hypertension, elevated blood lipids, liver disease, cervical arthritis, socio-economic factors,[14] migraine or arthritis.[2] This was most probably due to our study design and the small independent sample number (198 subjects). In addition, medical conditions like mastoiditis, meningitis, ear surgery, severe head and neck injuries and drug-induced ototoxicity were all exclusion criteria in the current study, and could not therefore be studied.

Familial effect

The mixed model approach showed a significant familial effect, even when accounting for known confounding factors such as hearing loss, gender and age. In order to estimate the importance of this effect we calculated familial correlations between siblings. The familial correlation for tinnitus of 0.16 was lower than those reported for ARHI. Gates *et al*.[25] calculated sibling-sibling correlations in subjects with a sensory presbyacusis phenotype between 0.39 and 0.17. This indicates that, although a familial effect was present, the effect for tinnitus is probably smaller than the familial effect observed in ARHI. This could be due to independent psychological factors such as personality and fearfulness,[15] which are thought to influence tinnitus.

The familial effect observed in the present study could be due to several factors of either genetic or environmental origin. If genetic factors influence the occurrence of tinnitus, these could be responsible for the familial effect that we have observed. The environmental factors can be attributed to a shared familial environment. All siblings in the present study grew up in the same family environment and probably shared a significant number of habits. All the siblings in individual families will therefore have been exposed to a common set of environmental factors. Some of these factors may play a role in the occurrence of tinnitus. So even though they are not genetic, they do contribute to the familial aggregation of tinnitus we observed in our study.

Possibility of genetic factors

Considerable familial aggregation was observed for ARHI.[25] Several other studies showed that this familial aggregation is largely attributable to genetic factors.[26-29] The proportion of variance in a phenotype that can be explained by genetic variances is known as the heritability of a specific condition. One study dealing with Danish twins specifically addressed the contribution of the shared familial environment and found no significant effect, although a modest effect could not be excluded.[26] By contrast, the ARHI consortium and other researchers have identified several genetic polymorphisms that are associated with ARHI.[30-32] For tinnitus, however, no specific causal genes have been identified yet, although Tyler *et al*.[15] have suggested the possibility of a polymorphism in the serotonin transporter gene (5-HTTLPR), which is associated with depression[33] and could influence tinnitus.

Possibility of shared familial environment

Several possible factors are related to a shared familial environment. In the case of tinnitus, several investigators have shown that a family history of either tinnitus or hearing loss has a significant effect on tinnitus annoyance and distress. Chéry-Croze and Thai-Van[34] showed that a majority of subjects with a family history of tinnitus or hearing loss reported an effect on tinnitus annoyance and on the impact on the individual's life. There have been reports of both positive effects, e.g. having a better understanding of the disorder or having a role model who has learned to live with the tinnitus, and negative effects, e.g. being afraid of deterioration in the future or of psychiatric side effects. In a different population, Kennedy and Stephens[35] confirmed these effects, showing that subjects with a positive family history of tinnitus and/or hearing loss were less affected by tinnitus in terms of 'annoyance', 'peace of mind' and 'enjoyment'. However, the effect in individuals was both positive and negative, mainly depending on the attitude the family member adopted towards the tinnitus.

Furthermore, we can postulate that the presence of a family member with tinnitus increases overall awareness of the phenomenon of tinnitus, and therefore increases the apparent prevalence of tinnitus in other family members.

Other possible factors might be a familial effect in environmental noise exposure e.g. workplace noise or recreational noise, or a familial effect associated with dietary patterns. There is little evidence for the first two factors. It has been observed, however, that a shared familial environment has a considerable effect on dietary habits.[36] The possible influence of diet on tinnitus may be caused by differences in dietary supplements associated with tinnitus, such as vitamins and minerals. Possible candidates include vitamin B12 and zinc.[37]

Further research

In the present multi-centre family study, we have shown that, in otologically normal subjects, there is significant familial aggregation in the perception of tinnitus. Several authors have already postulated the possibility of a genetic factor influencing tinnitus.

The familial effect observed in this study was lower than the effect found in age-related hearing impairment. There are several reasons for investigating this finding in more detail. Firstly, the differentiation between genetic and shared environmental factors could greatly enhance the elucidation of the exact pathophysiology of tinnitus. It would give direction to further research, pointing it either towards the shared familial environmental factors influencing tinnitus or to genetic factors, which will require specific genetic studies. In the first case, more emphasis could be laid on the impact of role models or attitudes that influence the perception of tinnitus. If genetic factors were identified, clinicians would have the opportunity to personalise treatment and counsel tinnitus patients. Differentiating between a patient in whom there is a genetic predisposition for tinnitus and a patient in whom this predisposition is not present could fundamentally change counselling and possible treatment for these patients. In the first case, gene therapy may be a possible approach, or a specific medical treatment might be advisable. In the second case, more emphasis may be laid on avoiding specific environmental factors.

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Appendix 1: Inclusion and exclusion criteria for subjects in the linkage study by the European ARHI consortium

Pathology	
1. Cardiovascular disease	
a. cardiac disease	Included
I. coronary disease	Included
1. angina pectoris	Included
2. infarction	Included
3. percutaneous transluminal coronary arteriography	Included
II. congenital heart abnormalities	Included
III. cardiac valve pathology	Included
IV. arrhythmia	Excluded
V. cardiac failure	Excluded
VI. cardiac transplantation	Excluded
b. pathology of the carotid arteries	Included
I. stroke TIA/CVA	Excluded
II. carotid surgery	Excluded
c. pathology of the femoral/popliteal arteries	Included
d. pathology of the abdominal arteries	Included
e. pathology of the renal arteries	Included
i. renal hypertension	
f. hypertension	
I. primary	Included
II. adrenal hyperplasia	Included
g. hypercholesterolaemia	Included

2. hormonal/metabolic disease	
a. hormonal	
i. diabetes	Included
1. type I	Included
2. type II	Included
ii. thyroid disease	Included
1. hypothyroidism	Included
2. hyperthyroidism	Included
iii. hyperparathyroidism	Included
iv. other	Excluded
1. Cushing's disease	
2. Addison's disease	
3. acromegaly	
4. hyperprolactinaemia	
5. diabetes insipidus	
6. phaeochromocytoma	
b. metabolic	
i. osteoporosis	Included
ii. renal disease	Excluded
1. chronic renal insufficiency	
2. hemodialysis	
3. transplantation	
iii. liver disease	
1. haemochromatosis	Excluded
2. cirrhosis	Excluded
3. chronic liver failure	Excluded
4. liver transplantation	Excluded
5. Gilbert's disease	Included
3. Autoimmune diseases	Excluded
a. rheumatoid arthritis	
b. lupus erythematosus	
c. inflammatory bowel disease	
d. ankylosing spondilitis	
e. temporal arteritis	
f. gout	
g. Cogan's disease	
h. Bechet's disease	
i. Wegener's granulomatosis	
j. IgA nephropathy	
k. Takayasu's disease	
l. polyarteritis nodosa	
m. scleroderma	
n. dermatomyositis	
o. Sjögrens disease	
p. other	
4. Neoplasms	
a. in the ear	Excluded
b. with therapeutic radiotherapy onto the region of the ear	Excluded
c. with therapeutic chemotherapy	Excluded
d. haematological neoplasms (leukemia, lymphoma, Hodgkin's disease, polycythemia vera, Waldenström macroglobulinaemia, amyloidosis	Excluded
e. brain tumours	Excluded
f. metastatic neoplasms	Excluded
g. other: local neoplasms	Included
General: no head and neck cancers. No chemotherapy, no local (head) radiotherapy	

5. Neurological disease	
a. (Alzheimer's) dementia	Excluded
b. Parkinson's disease	Excluded
c. multiple sclerosis	Excluded
d. epilepsy	Included
e. migraine	Included
f. other	Excluded
This implies all neurological diseases EX, except epilepsy and migraine	
6. Psychiatric disease	
a. severe mental illnesses for which hospitalisation has been necessary	Excluded
b. other	Included
7. Pulmonary disease	
a. COPD/asthma/emphysema	Included
b. pneumoconiosis – asbestosis - silicosis	Included
c. idiopathic pulmonary fibrosis	Excluded
d. cystic fibrosis	Excluded
e. sarcoidosis	Excluded
8. Haematological disease	
a. sickle cell anaemia	Excluded
b. haemophilia	Excluded
c. Von Willebrand's disease	Excluded
d. anaemia	Included
e. any haematological disease for which the subject is being treated	Excluded
9. Dermatological, ophthalmological, gynaecological disease; diseases of the stomach or bowels	Included
10. Infectious diseases	
a. AIDS	Excluded
b. syphilis	Excluded
c. Lyme's disease	Excluded
d. hepatitis B or C	Excluded
e. tuberculosis	Excluded
f. meningitis	Excluded
g. herpes Zoster	Excluded
11. Other conditions	
a. all congenital syndromes (Down syndrome etc.)	Excluded
b. all rare diseases that are severe enough to cause significant handicap according to the subject	Excluded