Minutes WG4 Genetic meeting, Stockholm 2014

I) Short summary meeting WG4 Genetics, October 24th, 2014 - Stockholm (Sweden).

The first meeting of the Genetics Work Group 4 from TINNET was held in Stockholm, at the Karolinska Institutet. Eight participants attended this meeting representing Sweden, Spain, Germany, Greece, Denmark and the Netherlands. The group members developed a step-wise approach to tackle the challenging aspects of understanding tinnitus in its genetics origins. This included a plan of action for addressing ethical issues, legal aspects, the design of the research studies, and finally the strategies for successful research funding by the EU.

List of attendees	Member	CO	E-mail	Attended
Thanos Bibas	mc	GR	thanosbibas@hotmail.com	Yes
Lennart Bunch		DK	lebu@sund.ku.dk	Yes
Christopher Cederroth	mc	SW	christopher.cederroth@ki.se	Yes
Rilana Cima	mc	NE	r.cima@maastrichtuniversity.nl	Yes
Jose Antonio Lopez Escamez	mc	SP	antonio.lopezescamez@genyo.es	Yes
Paul Van de Heyning		BE	paul.van.de.heyning@telenet.be	No
Marlies Knipper		GE	marlies.knipper@uni-tuebingen.de	Yes
Birgit Mazurek		GE	birgit.Mazurek@charite.de	Yes
Agnieszka Szczepek		GE	agnes.Szczepek@charite.de	Yes
Total				**8**

II) Agenda:

8:30- 9:00 Welcome-Registration

1. Welcome to all participants/introduction

9:00-11.00 Agenda discussions

1. Adoption of the agenda
2. Procedure for reimbursement of travel expenses
3. Revise central tasks for WG4 – Appendix A
4. Feedback from WG1 (clinical) and WG2 (database). Availability of samples and recruitment strategy.
5. Define strategies for genomic studies:
    A) Considerations on tinnitus subtyping
    B) Candidate gene approach
    C) Genomic approach

11:00-11:30 Coffee break

11:30-13:00 Agenda discussion

6. Proposals for genomics research studies

4.1. Exome sequencing in multicase families with tinnitus- development of an iPSCs-based model of tinnitus

4.2. Genotyping of target candidates in a tinnitus family

13:00-14:00 Light Lunch

14:00-15:30 Agenda discussion

7. Plan of Action

7.1. H2020 candidate topics: preselection of topics

7.2. Proposals to organize a Consortium for H2020

7.3. Potential subjects for a H2020 grant

15:30-16:00 Coffee break
16:00-18:00 Agenda discussions

8. Budget plan
9. Next WG4 meeting

III) Discussions

Main problem: there is very little knowledge on the genetic basis of tinnitus. Even, on a broader scale, we do not know if some populations are more prone to it than others, which could have argued in favor of a genetic contribution to prevalence. How to initiate such studies? How to coordinate between various institutions an efficient genetic study? We have found that countries in Europe differ a lot in terms of legislations on performing human studies (ethics), in the way of sharing human material or information, on the way of protecting the institution from legal problems that could be raised with the handling of biomaterial (insurance). Tinnitus is very heterogeneous and its complexity would dilute relevant genetic variants that would finally be unidentified. Finally, genetic studies are very costly - the more precise, the more information can be gathered by at a significant cost.

The following questions have been raised:

- What are the legal requirements of the EU state and the institution regarding sharing human material?
- What local insurances protect institutions from handling of DNA material?
- What type of studies can be designed in an early and late phase?
- How to obtain successful funding?

IV) Understanding of the genetic basis of tinnitus

Description of the genetic approaches

1. Candidate gene approach: This is an old-fashioned approach. It was based on the hypothesis that there is a strong knowledge on the biochemical bases of tinnitus (which is not the case). We could adopt this approach for a specific subtype of tinnitus (i.e., Kv7.2/3 in noise-induced tinnitus), but this strategy is too risky.
The association studies required a validation in a second cohort - almost impossible to do.

2. Genomic approach: no a priori biological hypothesis. Candidate single nucleotide variants (SNV) are screened throughout the entire genome. The more homogeneous tinnitus phenotype, more chances to find associated variants.

2.1. Genome wide association study (GWAS). Based on the hypothesis that many common SNV contribute to tinnitus. Genotyping SNV in a microarray. This is lot of money, large n=10.000. Price around 300.000€ for 10.000 samples. We need also controls from the same population. It takes years to recruit 10000 samples. We could determine risk markers with low size effect, so resolution is low.

2.2. Whole exome sequencing (WES) increases resolution. Exome (all exons in human genome). Price only WES (1.000 €/sample), if we plan whole genome

sequencing -intron included- is around 20-30.000€ per person. The more samples better but at least 100 person.

2.2.1.WES in multicase families: a successful approach to discover rare variants (MAF<0.01) with high penetrant effects. We need familial controls with the same phenotype BUT WITHOUT TINNITUS. We need to identify families in clinical centers.

Problem: not the same insult, rare to obtain with similar trauma.

2.2.2.WES in the extreme phenotype using a case-control design. We can select unrelated individuals with persistent, disabling tinnitus (TRI score > percentile 80) developed after an otological disorder (i.e., Meniere's disease, sudden SNHL, NIHL) and compared them with another set of patients with the same ontological condition without tinnitus.

3. Epigenomic by a twins study. We may have access to the Swedish monozygotic cohort (60000) Monozygotic, matched hearing loss with/wo tinnitus for epigenomic study. Price is around 10.000 for 12 samples.

Learning from previous research in fields like pain and schizophrenia, that are very heterogeneous disorders, we agreed to restrict the studies to the most homogeneous groups in terms of aetiology, age, gender balance, severity of tinnitus, audiometric profile. The smaller the genetic variance within a group, the more robust the study will be: studying twins > families with multicase tinnitus > groups with cisplatin-induced tinnitus following chemotherapy, noise-overexposure (military training or work exposure), or ARHL can provide useful information. As an example, Genome-Wide Association Studies (GWAS) of more than 100'000 people throughout Europe identified several important loci for schizophrenia (Ripke S., Neale BM., Nature, 2014 - 511(7510):421-7), whereas Whole Exome Sequencing (WES) of a more restricted but homogeneous cohort (age, onset, phenotype) of less than a hundred people identified one gene for schizophrenia further validated with mouse studies (unpublished results). Two strategies have been adopted to identify relevant subgroups of tinnitus individuals for genetic studies: i) using already characterized out-patients from clinics such as those from the Charité where homogeneous tinnitus phenotypes and/or families can be identified, ii) using cohorts from epidemiological health studies such as those that have already been performed in Sweden (Svensson CA., Int. J. Epidemiology, 2013 - 42:1263-72) where individuals that mentioned experiencing chronic tinnitus can be identified and further contacted for tinnitus scoring and in-depth auditory assessment. A standardized assessment protocol should be used in all clinics to facilitate the merging of multiple datasets.

Sources of funding: H2020 has emerged as an obvious source of financial support, although it was acknowledged that it would never cover all logistic/employment costs locally. Additional funding sources are thus needed. In 2015, no calls adapted to our aims has been found, hence we should wait for the 2016 calls to evaluate the possibility of submitting an application. In this direction, a letter of an expression of interest by the various members of TINNET send to Brussels H2020 administration could favor the occurrence of funding calls applicable to tinnitus.

Prerequisites for H2020 funding: Local information meetings on H2020 in Stockholm have emphasized on the need of providing evidence of a fruitful consortium, with already established agreements, and preliminary data. For this purpose, the WG4 has agreed in writing a first review on the current knowledge on the genetic origins of tinnitus, and also initiate a first study based on either twins (if available) or multicase families. In this regard, two multicase tinnitus families have been identified in Sweden (Cederroth) and their characterization is ongoing. Local funding for WES has been applied (Lopez-Escamez/Cederroth) with decision in early January. This could serve as a basis for either publication or preliminary data on a H2020 grant application.

Brainstorming on a potential grant structure based on the available group expertise: Christopher Cederroth suggested a preliminary grant structure gathering all participant's expertise to provide a multidisciplinary approach to understand tinnitus. The project is structured in two major tasks: i) understanding the genetic basis of tinnitus in humans and validate it with animal studies, and ii) translating knowledge from animal studies to humans. The first task is the core of WG4, in which identified variants may be further validated by experimental groups (Cederroth/Knipper).

Based on animal genetic studies, one candidate gene involved in tinnitus has been identified and opens possibilities of developing drugs to prevent/cure from tinnitus. A platform to perform such studies is available, and the proposal includes the testing of a device to quantify drug efficacy in humans.

The following structure is proposed:

A) Understanding the genetic basis of tinnitus in humans and validate it with animal studies

a. Genetics: WES, GWAS (Lopez-Escamez/Cederroth, Maruzek/Szczepek, Bibas, Van de Heyning, Cima)
   Population: Families, cisplatin-induced tinnitus, ARHL
   BioBanking (example of cost at KI: 26'000 € / 3'000 samples - DNA extraction included)
b. Validation with animal models

B) Translating knowledge from animal studies to humans

a. Mouse gain/loss of function specific to ear or brain (Cederroth, Knipper)
   Rat systemic gain/loss of function (Cederroth, Knipper)
   Validation in humans (genotyping – Lopez-Escamez)
b. Chemistry studies (Cederroth, Bunch, SciLife Labs)
c. Development of drugs: tool compound, ADMET studies, proof of concept (Cederroth, Bunch)
d. Biochemistry studies (unidentified partner)
e. Tinnitus diagnostic in humans (Cederroth, Ivansic-Blau)

Two companies have expressed interest in this work: Sensorion Pharmaceuticals (drug development) and Otometrics (tinnitus diagnostic). This should increase the value of the application.

Essential questions that need to be answered during the coming year:

- is the gene expressed in humans (Knipper, Bibas)?
- can already existing homolog drugs target the relevant organ (Cederroth, Bunch)?

V) Plan of Action

The following tasks will have to be addressed during the coming year:

1- Gather legal/ethical regulations for sharing biomaterial in EU

- identify the local legal ethical regulations, at the level of the institution and at the level of the state, on how to handle data coming from a foreign institution, and how to handle samples or patient information to be sent to a foreign institution.

2- Gather information on local liability insurances

3- Local Bionbanks: a centralized EU Biobank is not viewed as an option, because it would require complex agreements. The group has decided to identify local Biobanks.

4- Draft a consortium's agreement including non-disclosure for sharing information and data

5- Write a review on the existing knowledge about the genetic basis of tinnitus. Authorship as agreed during the meeting = First (Lopez-Escamez), Last (Cederroth), Middle (all). Journal aims: high. Lancet, Progress in Neurobiology

- Search strategy and classification of studies (Bibas, Cima)
- Epidemiological data (Cima, Bibas)
- Genetics (hearing loss, tinnitus) (Escamez)
- Behavior markers (Cima)
- Epigenetic modifications (Mazurek/Szczepek)
- Animal studies, candidate genes (BDNF) (Cederroth/Knipper)
- Pharmacology (Bunch)

Deadline: 29 January

First draft: 15 February (Lopez-Escamez/Cederroth)

6- Identify which would be the most prevalent/abundant tinnitus sub-type? The availability of samples will determine genomic studies.

7- Initiate a genetic study with available material:

- identify at least 5 families in available institutions, with 3-4 cases of tinnitus, and at least one control person without tinnitus.

8- Identify additional cohorts:

- contact local clinicians (urologists, oncologists) to identify potential groups with cisplatin-induced tinnitus
- contact already existing DNA collections from prevention studies (before disease onset)

9- Standardizing tinnitus assessment for the classification of tinnitus sub-types

- revise current TSCHQ, THI, TBF-12, Tinnitus Severity, ICD-10, WHOQOL-Bref
- include behavior related questionnaires
- include high frequency (hf) audiometry, hf pure tone ABRs, DPOAEs

10- Gather information and list local national grants sponsoring genetic studies of disease.