Database(d) Guidelines: A Small World

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The European Thyroid Association (ETA) has engaged itself in a mandatory though difficult task: the production of guidelines.

Mandatory because it is the duty of a scientific medical society to contribute to the education and training of the community and, based on updated knowledge, to help the clinician make the appropriate decision in common as well as in rare, sometimes complicated cases. Difficult because the way to guidelines, although paved mainly with safe cobblestones (high level of evidence issues), includes some uneven chippings (low level of evidence issues). Therefore, some international guidelines for the guideline developers have been produced [1]. Yes! Guidelines for guideline developers! Actually, what was proposed by Guidelines International Network is a hand-out of key components of high quality and trustworthy guidelines. They deal with the method for constitution of the guideline-developing group and nomination of the chair, the method of data analysis, the quantification of effect, the scoring system, the way of reaching a consensus upon debated issues, the conflict of interests, the scope of the guidelines, the way they will be discussed in the community (external group discussion), the way they will be implemented, the expected length of validity. All these key points have been created in order to avoid any unintended bias introduced by experts of the topic.

Personally, I would place the issue of the scope of the guideline in the first line because the disease or the condition on which the development group will focus largely determines the way some of the key points can be achieved.

The scope, here, is the familial and sporadic non-autoimmune hyperthyroidism caused by TSH receptor germline mutations [2]. This is a very rare disease, as stated in the introduction, which precludes any suitable assessment of prevalence, although the frequency was estimated to be around 1 in 121 in juvenile thyrotoxicosis in a Danish nationwide survey [3]. Nevertheless, the few clinicians, researchers, heads of genetic diagnostic laboratory, endowed with any kind of self-made expertise have been asked by colleagues, formally or not, for help in diagnosis and therapeutic and genetic counseling. A collective statement on the state of the art in the field is thus very welcome, and the ETA and the taskforce of these guidelines should be thanked for their efforts. Especially because only 27 families and 15 cases with sporadic form have been published to date, which makes any statement risky. Not all cases were published, obviously, and maybe the silent searchers should be blamed for their reluctance to just add another new case, either with a known mutation, or a new mutation with no sexy publishable characteristic to a not so long list. Indeed, any new case pub-

lished may in some way change the face of the syndrome. Let's examine some of the key points for guideline production mentioned above.

Unbiased Discussion

We live in a small world, as David Lodge [4] would say, where we know each other. The members of the taskforce and the chair are known; their representativeness is not questionable; there is no financial conflict of interest. Several members, including the chair are actively involved in molecular diagnosis and characterization of mutations of TSH receptor which is the basis of their expertise. It may, at most, lead to a biased view of some aspects.

Consensus

Since there is no indication of the way the consensus was reached, one should understand that either it was reached in informal process (more prone to biased results), or that there was no discrepant view. The latter hypothesis indicates, then, that either there was no need for guidelines if everybody, in our small world, thinks the same way or that recommendations may be biased.

Strength of Evidence and Recommendations

The readers must have understood that 27 families and 15 sporadic cases give no ground for more than an expert opinion. Quantification of the magnitude of benefit and harm is not possible here. Most of us, indeed, will agree with the recommendation of early total thyroidectomy and radioiodine ablation, but I do not think there is any strong evidence of a benefit of such attitude justifying a strong recommendation. Especially because the expression of the disease is highly variable in a same family, because total thyroidectomy in a child may cause more harm than good, and that a follow-up of thyroid hormones in asymptomatic carriers may be an acceptable option. In the same view, I am not convinced, as a clinician, that screening for a mutation in asymptomatic children should be strongly recommended (except for sporadic neonatal hyperthyroidism) if it is not sure there is some urgent action required. Different countries have different regulations for genetic testing which is not allowed everywhere in a child in absence of expected short-term benefit for the child him/herself.

Reviewing Process

Was there any external reading group? According to the ETA guideline rules, the draft of the guidelines was displayed on the website for one month for comments and discussion. Also the referees solicited by the *European Thyroid Journal* somehow play this role. However, their comments, accepted or not, their suggestions implemented or not, are not public as the reviewing process is anonymous, even in a small world. Actually, making public the correspondence between authors and referees, though keeping them anonymous, as is now the rule in some journals, might be a way to improve the validity and acceptance of guidelines.

Guidelines or Expert Opinion?

Guidelines are helpful and, when clearly and simply written, soon become the tables of the law, especially when read by readers unaware of their limits. Therefore, because of the preceding comments, I would suggest these recommendations be renamed 'expert opinion' or 'expert consensus'.

One difficulty in elaborating this consensus, I believe, and in commenting on it, is that it relates to two different fields: clinical guidelines on the one hand and (research) laboratory good practice on the other.

Analyzing the Data

Written sentences, especially published papers are practically engraved in the marble even if they only reflect the opinion of a limited number of researchers. The consensus proposed here largely refers to the Leipzig TSH receptor mutation database, a very valuable tool, offered to the community and recently updated, which is the topic of a second article in this issue of the *European Thyroid Journal* [4]. Indirectly, this exclusive referral to the Leipzig database can lead to a kind of silent ratification of the Leipzig view by all the authors and by the ETA, which has to be debated. Although small, our world is diverse, and actually three TSH receptor mutation databases exist. The Leipzig database [5], dedicated to TSH receptor including clinical features and a sorting of mutations, the Brussels database [6] and the Berlin database [7], the last two dedicated to the three glycoprotein hormone receptors (TSH, FSH, LH/hCG receptors), which may be an interesting tool when analyzing

Fig. 1. Faked presentation of cAMP as a function of cell surface expression for different mutants, based on published data, personal unpublished data, and expected behavior of hypothetical mutants. WT = Wild-type receptor; 1 = constitutively active mutant; 2 = extremely low surface expression and high cAMP production; 3 = higher cell surface expression than the wild-type receptor but identical slope. Please note that the experimental data leads to extrapolated aberrant different origins of curves.

a new mutation by providing some easy comparisons and recording the available data on each mutation. The three online sites <http://endokrinologie.uniklinikum-leipzig.de/tsh/>, <http://gris.ulb.ac.be/> and <http://www.ssfa-gphr.de/> provide links to the initial or related articles describing the mutation, thus helping the clinician or the researcher to make up his/her mind about the clinical features and the experimental data. Connecting to the three sites helps keeping an open mind when analyzing the published data.

Why? Because there has been some controversy about a few TSH receptor mutants that were described as constitutively active in initial reports, and found not to be constitutively active upon further assessment by another group [8–10]. The methods were not exactly the same, the cell lines were different, the way of assessing the membrane expression was different, To make it clear if not short, a mutant is said to be constitutively active when it leads, in absence of stimulation by TSH, to a higher production of a second messenger (mainly cAMP) than the wild-type receptor. This is measured in eukaryotic cells after transfection of a vector including the cDNA coding for the TSH receptor, that is, in a *very artificial overexpression* system.

Should Linear Regression Analysis Become a General Rule?

Because the mutants and the wild-type receptor may have a different level of expression at the cell surface, the cAMP production has to be normalized to the number of receptors to allow for valid comparisons. Constitutively active mutants have a higher production of cAMP per receptor unit. Because results were sometimes discrepant, it has been proposed to analyze the results by measuring the cAMP production in cells expressing increasing quantity of TSH receptor and plotting the cAMP as a function of receptor cell surface expression to obtain a linear dose response and to calculate the slope of the curve (fig. 1). It was claimed that a significantly steeper slope was the hallmark of a constitutive mutant (fig. 1 curve 1), and that mutants having a similar slope with wild-type receptor are not constitutively active mutants [10], and the guidelines recommend using this slope for borderline mutants. This way of analyzing the *in vitro* results, however, may largely depend on the way the cell surface expression is measured. This should certainly not be by binding experiments, as the affinity for the ligand is, first, different for the mutants and the wild-type receptor, and second, it decreases when the level of expression

increases even for the wild-type receptor. Whether this is due to an artifact or to negative cooperation is beyond the scope of this editorial. I would, thus, not bet a penny on a slope value determined this way, even for mutants with very high constitutive activity. Some of the mutants analyzed this way display such a low binding that the reality of it is highly questionable (fig. 1, curve 2), and was even considered to be absent for one [9, 10].

Interestingly, a mutant with a higher cell surface expression and the same cAMP production per unit of receptor, thus a higher rough cAMP production, would not be regarded as constitutively active because of an identical slope (superimposed but elongated curve; fig. 1, curve 3). Especially if the higher cell surface expression was observed also in vivo in thyrocytes, this mutant would, undoubtedly, lead to hyperthyroidism.

Two concepts are mixed here: activating mutations and the so-called constitutively activating mutations. The first one would relate to the final in vivo end point: higher production of cAMP and of thyroid hormones – this is the one relevant for the clinician managing a patient or a family. The second would relate to a molecular feature: higher production of cAMP per cell surface molecule of the TSH receptor.

Finally, this normalization to the cell surface expression of the receptor overlooks recent data indicating that the TSH receptor signalizes also from the intracellular compartments [11, 12]. The accepted normalization of cAMP production to the cell surface, whether in a linear regression analysis or not, may thus be one but possibly not the best way to calculate the activity of the receptor, at least for some mutants.

Beyond this expert debate, the only solid recommendations for this topic would in my view be

- (1) That the mutation, when not previously fully described, should be carefully characterized and that borderline results should be interpreted with caution.
- (2) That the clinical and familial context should also be part of the decision to consider it as activating or not.

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Practically, the mutation described as constitutively active in vitro but identified in a family with resistance to TSH may provide an interesting activation model for molecular studies, but it would certainly not be regarded as a clinically activating one. On the other hand, one mutation for which the constitutive activity was questioned by the Leipzig group [10] was recently identified by the same group in another family with hyperthyroidism [13] despite serious doubts on its constitutivity raised according to the linear regression analysis. Where should it be placed in the Leipzig database? Activating mutation, as in the Berlin database? Neutral mutation? Questionable mutation? The latter category does not exist in the database. I would suggest, in order to reconcile divergent data and to acknowledge some uncertainty, when guiding the reader, to create a ‘pending assignment’ category.

Again, the authors should be sincerely thanked for their endeavors to propose some references to the clinicians. However, low level of evidence, uncertainties, debated questions and issues cannot be overlooked and, in my mind, do preclude any strong recommendation, either regarding the clinical management of the patient or the in vitro characterization of the mutants. Should we rename the guidelines, I vote for ‘expert opinion’.

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