First of all, I would like to thank Professor Lanzi and Professor Aïcardi for having invited me to this very interesting meeting – not interesting in number, maybe, but interesting in the form of the passion that we all share for this syndrome, be it the active players, parents or just friends like me.

I must advise you not to expect any breakthrough research results in this presentation. As you have heard, I am a biotechnology consultant and my objective is to present the prospects and the technical possibilities that have been opened by the latest developments in the pharmaceutical industry, after the sequencing of the human genome.

The title of my presentation is “Rare Genetic Diseases - New Opportunities and Challenges through Biotechnological Progress and Scientific Knowledge”. As you heard, I am a consultant to a company called Gene Control SA, and it is not very difficult to guess the activity of Gene Control: It's a gene therapy company. Today, with respect to the Aïcardi-Goutières Syndrome, as with other very rare genetic diseases, it is premature to discuss the benefits and potential of gene therapy procedures, but hopefully one day we will.
Rare Genetic Diseases

To start this talk about rare genetic diseases, I need to go back where it all starts, and that is the chromosomes, our genetic inheritance.

The above picture shows the chromosomes of a human cell. Each of these chromosomes is organised in a precise sequence of genes and a special function is assigned to each gene. There are 23 pairs of chromosomes in the human genome, which makes a total of 46 chromosomes, including the X and Y so-called sex chromosomes.

On each chromosome we have a certain number of loci and today the complete human genome comprises more than 7,000 loci.

Progress in human genome research is registered in genetic data banks like the ‘On-line Mendelian Inheritance in Man’ that can be accessed through Internet. To date some 12,000 loci have already assigned functions, genes or phenotypes. These data are increasing every day. Estimations of the total number of genes of the entire human genome are conflicting and range from 30,000 up to 100,000 genes. Even with the lowest estimate, it is clear that hunting for a genetic error in this huge amount of genetic information becomes a gigantic task.

Let me go back a little bit more deeply to what I would call ‘from gene to therapy’ and the fundamental dogma postulated by Nobel prize Francis Crick:

\[
\text{one gene} \rightarrow \text{one protein}
\]

Normally, one gene leads to one protein, through the transcription of DNA into a messenger RNA molecule, which in turn translates into a protein:

\[
\text{transcription} \quad \text{translation} \\
\text{DNA} \rightarrow \text{mRNA} \rightarrow \text{Protein}
\]

Generally speaking, it is the protein that has the final biological activity. So, this means that, if we have an impaired gene function, it will lead to either abnormal proteins, non
functional proteins or to a protein imbalance. For example, a high level of interferon-alpha is a protein imbalance.

|| impaired gene function
|↓|
| ☐ abnormal proteins
| ☐ non-functional proteins
| ☐ protein imbalance

Impaired genetic sequences, that can arise from all sorts of damage to or interference with DNA, eventually lead to disrupted protein homeostasis. Unbalanced protein homeostasis is a change from the natural situation to an unnatural situation and can lead to pathological consequences.

|| impaired genetic sequence(s) - DNA
|↓|
| disrupted protein homeostasis
|↓|
| disease

So, when we look at a genetic syndrome, we have the choice to study either the proteins and their functions or we can take it from the genetic sequences of a patient and look for major damages or mutations in his genome. So, there are at least two different possible approaches that can improve the understanding of the mechanisms of a genetic disease.

In any case, just as for any other disease, there is a need for a multi-level approach to study diseases that originate from genetic malfunction. Why multi-level? First, the fundamental mechanisms of the disease need to be understood, a task to be carried out by research. Second, clinical diagnosis tools need to be developed for early and unambiguous assessment of diseased patients, preferably through non-invasive procedures. Last not least, a cure is sought to treat patients and the doors opened by the rapid progress of gene therapy technologies represent some space for optimism in that sense.

Regarding the fundamental research aspects of the Aicardi-Goutières Syndrome (AGS), progress is hampered by the lack of a meaningful animal model, even if today there is some hope that the Campbell model from La Jolla could be validated as a model for AGS. (Akwa Y, Hassett DE, Eloranta ML, Sandberg K, Masliah E, Powell H, Whitton JL, Bloom FE, Campbell IL. Transgenic expression of IFN-alpha in the central nervous system of mice protects against lethal neurotropic viral infection but induces inflammation and neurodegeneration. J. Immunol. 1998 Nov 1;161(9):5016-26. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA
Research in the field of AGS focuses on the genetic characterisation through gene mapping, locus linkage and on the study of pathological pathways and abnormal cytokine expression.

Clinical diagnosis, including prenatal diagnosis, needs a development boost, for example on phenotyping / immunotyping platforms, as demonstrated elegantly earlier by Doctor Kuipers, and his promising approach. Diagnosis tools may also be developed on the basis of biochemical markers like interferon-alpha levels or other biochemical mediators as they become identified. The utmost diagnostic tool would be the specific DNA sequence(s) containing the genetic misfit(s) that underlie AGS. In order to identify the responsible sequences, a fully-fledged genetic characterisation of the syndrome is needed. However, a success in that sense would also represent the key for possible cures of the diseased gene(s).

When we talk about therapy, in the case of AGS, we cannot see any clear straightforward route yet, as the principal disease targets still have to be identified and characterised. To date, all known AGS patients are treated on a case per case basis with a focus on the secondary effects of the disease. The only treatment that I know about is at the trial stage and it refers to the interferon-alpha reduction trial with metylprednisolone dosing.

Rare Genetic Diseases and Expectations

What can we expect from the biotechnology and life sciences progress today? There are new trends, new technologies and new opportunities, hopefully. But what are these new opportunities? Today the accomplishment of the human genome sequencing has generated great expectations in a number of fields, among them gene therapy, cell therapy and drug discovery in general. However, it is misleading to think that since the human genome has been sequenced, everything has been solved regarding genetic impairments. It is fair to state that the achievement of the human genome project will have repercussions in the field of rare genetic diseases like AGS, most probably through the identification of altered genes that lead to the onset of the disease, but the availability of the full human sequence represents also some major challenges. Indeed it is a toolbox that is so immense and complicated by all levels of interactions that misinterpretations arise easily and fundamental mechanisms may remain uncovered. For this reason, the big leaps promised by the genome-derived disciplines will happen only once bioinformatics tools will be able to cope with the pace of discovery.

A clear evidence that the human genome sequence is not an answer to all questions per se lies in the conflicting estimates of the total number of genes comprised in the complete human genome. The early approximation of 130,000 genes had become some 30,000 genes and now the figure is rising again.
Regardless the challenges that remain to be faced, thanks to the human genome project new disciplines have arisen that could be beneficially employed for the study of rare genetic diseases. More precisely, new areas of expertise, like genomics and proteomics, do help pharmaceutical companies to dramatically speed-up their discovery process for new disease targets, hence potentially new drugs:

“Genomics has changed our ability to identify new targets for drug action; we have gone from famine to feast” *SmithKline Beecham*

“Triple the number of drugs developed internally by 2000 ……… 3 major launches in the period to 2001” *Bristol Myers Squibb*

“The company plans to have 23 new drugs on the market by 2001” *Pfizer*

“Three significant new medicines per annum by 2000” *Glaxo Wellcome*

Now, if pharmaceutical companies are using these technologies to identify new genetic targets for major diseases with consistent market potential, what does hinder progress in dissecting the genetic basis of rare genetic diseases with the same swiftness? Probably, the reason is simply a lack of funding; drug development companies are above all interested in diseases that guarantee a decent return on investment; public funding is most often short and the few research groups involved in AGS, and rare genetic diseases in general, depend heavily on institutional funding and foundations like Telethon.

**Genomics**

Genomics encompasses the analysis of complex gene expression patterns, that is the messenger RNA transcripts present in a given cell. These patterns allow analysing the regulation of specific genes and detect any up- or down-regulation or the appearance/disappearance of specific gene transcripts (genes switched on/off). The strength and the power of this technology lies in the fact that there is the ability to analyse up to 30,000 genes almost simultaneously on gene chips, for example, and detect abnormal gene functioning in one run. There is the possibility to screen known and unknown gene sequences (ESTs – Expressed Sequence Tags), which is even more startling. One can imagine identifying an EST that has implications for AGS, but whose functions are not known or fully characterised. Analysing the function becomes an issue for what is termed functional genomics, i.e. the study of the biochemical and physiological role(s) of the identified sequence.
Regarding genomics tools, I must point out that, especially for neurobiology and neurochemistry, related gene chips are available; just as an example: the biotechnology company Becton Dickinson/Clontech from Palo Alto, California offers gene chips containing about 588 genes that have relevance to neurobiology. Running for example a patient’s RNA extract over such a gene array allows one to identify immediately the transcripts, that is the messenger RNAs transcribed from the genomic DNA, that are ‘abnormally’ regulated (overabundance or shortage).

Genomics indicates if there is an abnormal messenger RNA level, either too high, too low or just disappeared or appeared where it shouldn’t. But it doesn’t give any indication at the protein level, the real final biological effectors. One other trouble with genomics is that there is a need to identify the tissue, organ or the cells where to extract the messenger RNA. Is it neurological tissue that we have to take, is it blood, is it both, is it keratinocytes or whatsoever? Thus it becomes of utmost importance to define an accurate sampling strategy.

**Functional genomics**

Functional genomics is the investigation of the functional properties of a given gene sequence. As mentioned earlier, for many of the genes that are now sequenced we don’t know their functional properties. This is the reason why this new discipline has seen the light.

**Pharmacogenomics**

Pharmacogenomics is the study of individual susceptibilities to drugs, like aspirin for example, and this lies mostly in slight genetic variations called single nucleotide polymorphisms.

**Proteomics**

Proteomics means the analysis of complex protein patterns within a tissue or a cell. This technology allows determining which proteins are present in a cell of a healthy individual, and compare them with the proteins present in the cells of a diseased patient. Thus, proteomics allows to measure the appearance and disappearance of specific proteins, or the indirect measure of up- or down-regulation of specific genes.

Again, these technologies are very powerful and thousands of proteins can be analysed in one run on a daily basis. Also peptides and proteins with known and unknown functions do pop up in this kind of studies. Abnormal protein patterns, then, indicate abnormal gene functioning. And, if I push this forward, appearance and disappearance or up- and down-regulation of proteins and peptides gives an indication of where to look at the DNA, at the level of the gene.
### Summary table of genome-derived disciplines and major applications

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#### Towards a personalised medical care

Thanks to the disciplines described above, today’s trends in human therapy are moving towards individualised therapeutics, almost tailored according to the patients genetic asset. This means that technologies like genomics and proteomics are already actively being employed in the pharmaceutical sector, striving towards a future where it will be possible to elaborate a genetic ‘susceptibility’ profile for each individual before making a prescription. Moreover, these technologies could be beneficial for studying patients with AGS by establishing which genes are malfunctioning, which proteins appear abnormal, and so on.

#### The foreseeable future

I assume the statement that the gene or the genes involved in AGS will be identified in the not-so-far future. These data in hand should allow considering possible therapeutic strategies:
Pharmaceutical drugs that allow regulating genes that have come out of control, for example genes that have an implication in raising the interferon-alpha level; compounds that act as repressors or activators could be used profitably.

Ultimately, gene transfer will be an option to consider, if we can really identify a defective or a missing gene and transfer a healthy gene sequence from an healthy individual into the patient’s tissues.

Antisense technology is also a promising therapeutic tool which can be considered a therapeutic possibility for the future; briefly, antisense technology allows shutting down the synthesis of an undesired protein by acting on the messenger RNA.

When looking into the future, we must be aware that the medical and health environment is changing very rapidly, also due to the explosion of the information technologies. First of all, there is now a high degree of awareness at the consumer level, i.e. the patient himself. The pace of technology innovation in various fields is tremendous and, with the help of information technology, these technologies can be channelled towards efficient Research & Development in the context of genetic diseases.

Scientific advances and information availability: all knowledge-based information that is generated must be multi-layered, multi-structured as available data, so as to allow a real flux of information. Not everybody sitting in his corner and doing his own thing, without his neighbour knowing what he is doing.

But we must not forget that, if hopefully a gene or genes implicated in AGS are identified, it is still not said that by blocking or activating this gene(s) we have solved everything because things do always interact and the fine equilibrium that lies under the surface is sometimes difficult to rebuild.

But we must also remember that not only things interact, but we need to interact too. There needs to be a very tough communication scheme between all the people involved here, all the players concerned, because the possibilities for achieving consistent public funding are low and big pharma giants are not ready yet for stepping into the field of rare genetic diseases.

I thank the members of IAGSA for having organised this day because, without them, this would never have happened.