

When Genes Are out of Place

Even a small genetic error is sufficient to throw the highly organized work of nerve cells in the brain out of sync. In Germany alone, for around 2 percent of the population, the result is a mental disability.

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INSTITUTE FOR MOLECULAR GENETICS *in Berlin, is using DNA chips to search through the approximately 3.2 billion building blocks of the genome for the causes – and hopes this approach will also provide the basis for new therapies. At the same time, as he reports in this article, such research could show whether there actually are intelligence genes.*



Luminous chromosomes: Colored DNA probes highlight the various regions of the genetic material. In this image, chromosome 21 is present in three copies.

The human brain is by far the most complex construction in biology. The finely tuned orchestration of its many billion nerve cells gives rise to consciousness and intelligence. Innumerable cells and many specialized proteins are involved in conducting electrical impulses and thus also in learning and processing stimuli, and in predictive behavior.

If only one of these proteins is missing, the consequences can be dramatic: for example, some of the cell protrusions may no longer find their target or may no longer form

any permanent connections with each other. In the densely woven mass of neurons, holes and other imperfections can appear that profoundly affect the work of the brain. Other genes play a role in the modification of DNA or the breakdown of proteins – the list of possible causes is long.

In Germany, around 2 percent of the population live with an intelligence quotient (IQ) below 70 and are therefore considered mentally challenged; an IQ below 50 indicates a severe mental handicap, which af-

fects around 300,000 to 350,000 people. Around 8 percent of all health care contributions go to medical care and supervision of the mentally handicapped. The causes of these malfunctions are frequently unclear. Following the evaluation of numerous studies, many researchers now believe that the IQ range from 50 to 70, with its mild cognitive disorders, represents the lower end of the normal human intelligence distribution.

These handicaps can be traced back to complex interactions between many inherited and environmental factors. In contrast to this, people with severe mental retardation have one or even more errors (mutations) in their genetic material. Defects in thousands of genes can presumably cause disruption of brain function. Chromosomal changes can often be found, such as deletions or duplications that usually affect a number of genes, or chromosomal breaks that may occur in the middle of a gene, pulling it apart.

The best-known example of mental retardation is Down syndrome, also known as trisomy 21. Here, chromosome 21 (the smallest of the 46 human chromosomes) is present, not as two, but as three copies. Such a trisomy also exists for all the other chromosomes; however most of them lead to death as early as during embryonic development. The problem in Down syndrome is consequently too much intact genetic material, and not too little, as with chromosomal breaks or many mutations.

But how can too much DNA have such serious consequences? The reason for this lies in the complex control of genes. We now know that a

large part of our genome serves only to control the activity of other genes. A surplus of such genetic material can result in many other genes being switched off – with the well-known and serious consequences of Down syndrome.

Most severe mental disorders, however, are due rather to a defect in a single genetic trait. These monogenic diseases at least make the search for the cause a little easier, as only one individual gene defect has to be identified. Other health problems, such as diabetes and cardiovascular conditions, arise through the interaction of many genes and other factors, such as lifestyle, too little physical activity and the wrong diet, and not only through single, detectable mistakes in the genome.

SUCCESSFUL SEARCH FOR DEFECTS

Following the sequencing of the human genome, there was great hope that the causes for all frequent diseases could be simply read from the data – which turned out to be much too optimistic.

More recently, a shift in thinking has occurred (at least in the USA), and the investigation of inherited monogenic diseases has again become a central focus. Since the start of this research, around 1,700 different disease-causing gene defects have been identified in which the resulting inherited disorders are passed on to offspring according to Mendelian laws. The sequencing of the human genome has indeed facilitated the elucidation of these monogenic diseases.

For affected patients and their relatives, our knowledge about the genetics serves to explain the cause of the ailment. This information is of great benefit for many affected individuals in terms of helping them to

Photo: Focus-SPL

better evaluate and perhaps also better accept their situation. A large part of genetic counseling work is also based on this information. In addition, a correct diagnosis is of great importance as it can highlight alternatives to refraining from child-bearing. In Germany, about half of all genetic counseling concerns the prevention of hereditary cognitive disorders.

Basic researchers and medics hope, however, that there will one day also be new medications for treating mental disorders. Indeed, there are great expectations in this respect, since in the last few years it has been shown by my colleague Tobias Bonhoeffer, among others, and by researchers at the Max Planck Institute for Neurobiology, that the brain is not a hard-wired computer.

The making and breaking of contacts between nerve cells depends on environmental triggers, and it is a continuous process that may even involve the formation of new nerve cells. The brain thus remains plastic and flexible not only immediately after birth, but also into adulthood. If an explanation can be found for the metabolic processes that underlie these changes to the brain circuitry, drugs could, in principle, be used to intervene. Therefore, it is tempting to

speculate that, at least for some of the various genetically caused mental handicaps, drug treatment will be possible once the details of the underlying molecular mechanisms are understood. The example provided by fragile X syndrome indicates that these are not simply theoretical considerations: new experimental findings support the idea that there could be a medication for this disorder in the foreseeable future.

PATIENTS BENEFIT FROM BASIC RESEARCH

This would be a great advance. Around a quarter of all males with X-chromosomal forms of mental handicap have this condition. In fragile X syndrome, a region on the X chromosome is particularly prone to breaks that are associated with the loss of the functional FMR1 gene. This results in increased activity of specific receptors for the neurotransmitter glutamate in the patient's brain.

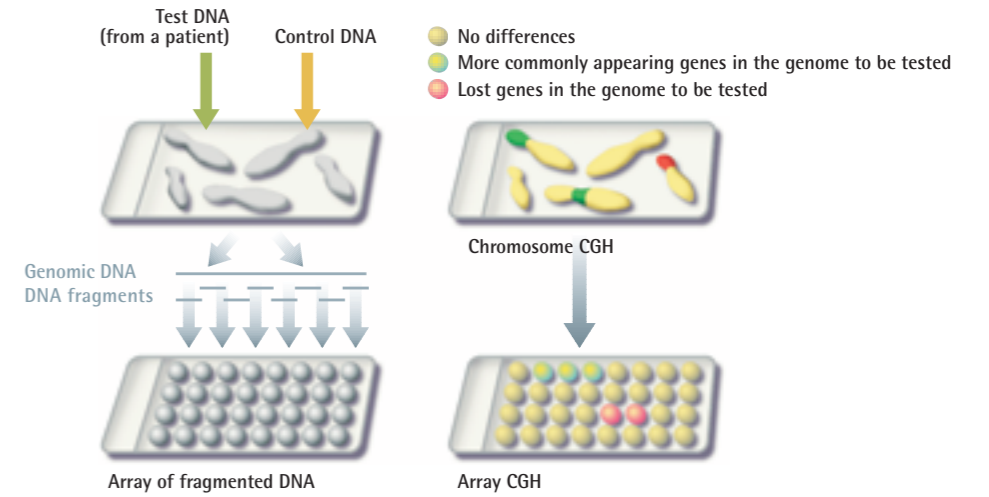
It was recently shown in adult fruit flies (*Drosophila melanogaster*) that memory loss associated with this gene defect could be relieved by drugs that regulate the over-activity of the affected glutamate receptors. This restored the short-term memory and normal mating behavior in the

insects. Similar experiments are also currently being performed in mice, and US companies are already exploring possibilities for the application of analogous procedures to treat fragile X syndrome in humans.

The task of searching through the approximately 3.2 billion building blocks of the human genome for often minuscule changes that cause mental retardation is the focus of research for a large group of geneticists, biologists and bioinformatics experts here at the Berlin-based Max Planck Institute for Molecular Genetics. For some time now, we have been searching for such gene defects by examining patients with "balanced" chromosome changes. Such rearrangements result from two

chromosomal breaks, followed by exchange of the resulting fragments. Due to the altered chromosomal structure, a balanced chromosome aberration can often be recognized under the microscope. Since no genetic material is lost or gained, most carriers of such changes are healthy.

However, in 3 percent of those affected by such aberrations, these chromosomal rearrangements do indeed lead to clinical manifestations: if the strand of genetic material, or DNA, happens to break within a gene, then this gene is lost. About half of these patients will then be mentally retarded. Mapping the chromosomal breakpoint region in such patients is therefore a very promising strategy for identifying



Comparative genomic hybridization (CGH) finds differences between the DNA from patients and healthy individuals. Conventional (chromosome) CGH employs normal chromosome spreads as a hybridization target, whereas for high-resolution array CGH, these chromosomes are replaced by sets of DNA fragments that represent the human genome. In both cases, a mixture of the green-tagged genetic material of the patient and the red-labeled control DNA are added. When a patient has many copies of a green-tagged gene, this will bind very frequently on the chip, forming a green spot. If a patient is missing a gene, only the control person's red-labeled DNA will bind. In the absence of dosage differences, superposition of red and green signals will yield a yellow color.

genes that are essential for normal brain function.

The work distantly resembles that of a clockmaker who first examines a defective and unfamiliar clock for the damaged part, and then at some point understands exactly how one tiny broken cogwheel can paralyze the whole clock mechanism. Our studies will contribute to a better understanding of the workings and development of the brain in general.

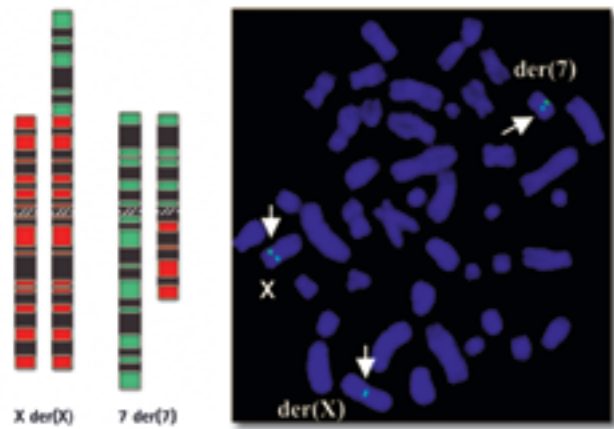
LUMINOUS PROBES EXPOSE BREAKS

In order to find the breaks, geneticists construct luminous DNA probes. These are made in such a way that they can associate with only a specific, known region of a chromosome: here, a colored spot appears under the microscope. In principle, such probes allow the 'painting' of each desired region of a chromosome, which makes it visible under the microscope - a tried and tested technique for many years now. In healthy people, there are usually two signals with such probes: they bind to both the paternal and maternal chromosomes.

Things are different if a chromosome, say the paternal one, is broken in the region of the probe. Then

there are three signals: the probe binds as before to the intact maternal region, but now it also binds to both halves of the disrupted paternal region. This procedure is called FISH (fluorescence *in situ* hybridization). With this method, we have mapped several hundred breakpoints over the last few years and found more than 30 different genes that are inactivated in patients with cognitive defects. Most of these genes are active in the brain, and for quite a few of them, there is already independent biochemical or neurological evidence that they play an important role in brain development or function.

Furthermore, we are now working on a new, highly exciting approach for elucidating the molecular aberrations underlying cognitive defects: the search for small deletions or duplications in the genomes of patients. The existence of such mutations is something that had not been reckoned with before now. At our institute, 36,000 human DNA probes, each with a length of about 150,000 base pairs, were fixed to precisely predetermined positions on a glass DNA chip in order to detect these kinds of changes. The DNA sequence and the position on the chromosome are known for each probe, and there



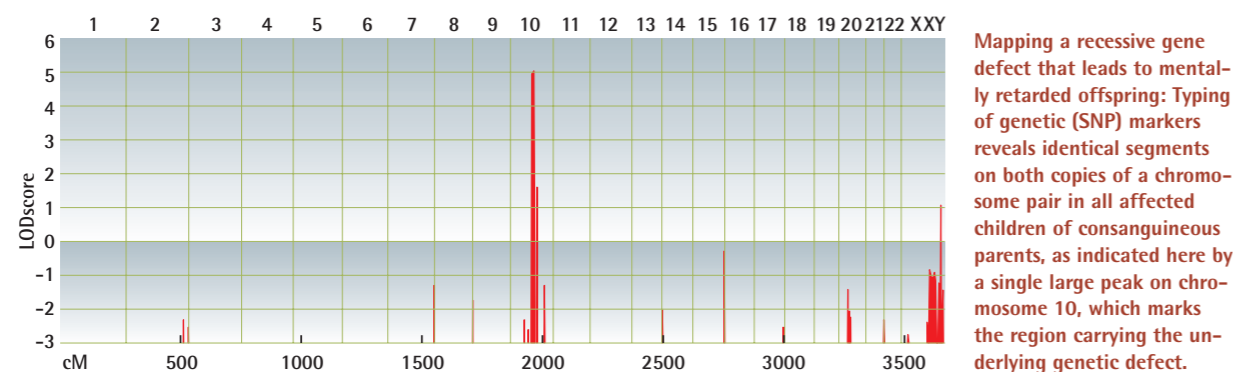
In this microscopic image, fluorescent spots (arrows) indicate the sites of chromosome breaks that have resulted in an exchange between chromosome X and 7. Signals are visible on both the rearranged chromosomes and the normal X.

In fruit flies, the consequences of a genetically determined memory loss can already be treated by drugs.



PHOTO: FOCUS-SPL / GRAPHIC: CHRISTOPH SCHNEIDER, BASED ON MATERIAL FROM THE MPI FOR MOLECULAR GENETICS

GRAPHIC: ROHBER, BASED ON MATERIAL FROM THE MPI FOR MOLECULAR GENETICS



is space for the whole genome on this chip.

The second step involves preparing the samples to be investigated: the DNA of a healthy person is broken up into smaller fragments and coupled to a red dye. The same is done to the patients' DNA, except that it is labeled with a green dye. For the test, the DNA from patients and from healthy control persons is applied to the gene chip carrying the total human DNA. There, the added fragments seek out their partner sequence. If a particular genome region is duplicated in the patient, more green fragments than usual bind to the DNA on the chip. Conversely, if one position on the chip shows a red signal, this indicates that the patient's DNA is missing a fragment of genetic material there.

Subsequent evaluation of the many thousand colored spots on the chips is done by machines. The interesting DNA regions in the patient's genome revealed in this way can subsequently be analyzed in more detail and, if required, sequenced down to the last base. With this information, in turn, we can look for similar defects in the genomes of other affected individuals. This means that we can reduce the problem in one step in an unprecedented way: instead of 3.2 billion base pairs, we now need to look for gene defects in only one individual fragment of around 150,000 base pairs – a fantastic step forward.

The technology behind gene chips is laborious. Experienced, trained

personnel and competent programmers are required to prepare the data and extract the desired information. The fluorescent dyes are very sensitive: particularly in the summer months, even the ozone has to be filtered out of the air or else the aggressive gas bleaches the dyes. In order to be able to interpret the data obtained, they have to be compared with all existing knowledge relating to the corresponding genome region and the disease in question. For this, access to international databanks is required. And without fast computers and intelligent software, this kind of research would be impossible.

ONE INTACT COPY IS OFTEN ENOUGH

Most patients with mental disabilities represent isolated cases. Particularly in western societies, families with more than one affected person are rare, and the possibility that isolated cases can also have genetic causes is frequently not taken into consideration. Aside from as yet unidentified small chromosomal changes, so-called recessive gene defects are likely to play a major role in such cases, but they are still largely unexplored.

For recessive inherited diseases to emerge, both the paternal and maternal copy of a gene must fail. If only one of the two gene copies that are passed on by the parents to their child is defective, the intact copy can provide the necessary protein, and the condition does not emerge. Practically everyone carries the predispo-

sition for one or the other recessive disease, and healthy relatives often carry the same gene defect. With children whose parents are related, an inherited disease may emerge more frequently – and this is always the case when they inherit the same disease predisposition from both parents. The low frequency of families with more than one mentally handicapped child in Germany can be explained by two factors: the very low birth rate, leading to very few families having several children, and the low frequency of marriages between members of the same family.

On the other hand, this explains why recessive inherited forms of mental retardation are still largely unexplored. To narrow down the responsible genetic disposition and finally identify it, geneticists require large families with as many patients as possible, which in western countries – luckily – is very infrequent. In other parts of the world, this is fundamentally different, and consanguineous parents are much more frequent. This has financial and cultural grounds; marriages are frequently arranged between members of the same clan. In Iran, for example, about 40 percent of all children's parents are related. In addition, the number of children per family is much higher than in Central Europe, which is also reflected in the fact that 70 percent of the population is younger than 30 years of age. Consequently, families with several mentally retarded children are considerably more frequent. For this reason,

we have drawn up cooperation agreements with Iranian research institutes in order to elucidate these autosomal recessive inherited cognitive defects.

Affected children from such a marriage of relatives inherit not only two identical copies of the same defective gene from their parents: the flanking regions on both corresponding chromosomes are also completely identical. Normally, such regions differ in a number of individual DNA building blocks; such single nucleotide polymorphisms, or SNPs, can be detected by various simple methods. The lack of any genetic differences between two chromosomal regions in patients is therefore quite conspicuous – and can be used to map recessive inherited gene defects in children whose parents are related.

HELPFUL COOPERATION WITH IRAN

Through agreements with our partners in Iran, where a nationwide network of genetic counseling already exists, we have access to a practically unlimited number of consanguineous families with severely mentally handicapped children. With around 70 million people, the Iranian population is almost as large as Germany's, and politicians there have recognized the significance of this research and strongly support it.

Our long-term goal is to identify the molecular causes of all the frequent forms of mental retardation. Using special funding from the Max Planck Society we have already examined more than one hundred Iranian families, and were able to localize a good dozen new genes in the genome, thereby increasing the number of mapped genes five-fold. A model for these investigations is a

successful European collaborative research program that aims to elucidate X-linked forms of mental retardation, to which we have contributed significantly in the course of the past decade. Today, we are able to make a defined molecular diagnosis in almost 50 percent of such patients – in the affected families, no more disabled children have to be born.

Research into the genetic, biochemical and cell biological basis of such defects will keep us busy for a long time. In many cases we will need to create animal models of such disorders, e.g. mice that are missing both copies of the relevant gene. And a particularly interesting aspect of this work is the question of whether and to what extent the many variants of these genes are responsible for the variability of intelligence in the normal population.

Perhaps this will tell us whether there are individual genes that significantly influence the intelligence of healthy people. In fact, this question seems to occupy the German press more than the research into causes of mental retardation, despite the associated enormous socio-economic burden for the affected families and our society as a whole. ●



PHOTO: MPI FOR MOLECULAR GENETICS

PROF. DR. HANS-HILGER ROPERS, born in 1943, studied medicine in Freiburg and Munich and was a scientific staff member at the Institute for Human Genetics at the University of Freiburg; he obtained his Ph.D. qualification in 1973. Following his German post-doctoral lecturing qualification he was appointed professor at the University of Freiburg (1981), became professor and head of the Department of Human Genetics at the University of Nijmegen (1984 to 1997), as well as board-certified clinical geneticist (1987). In 1994, he was appointed Director and Scientific Member of the Max Planck Institute for Molecular Genetics in Berlin.