ETHICAL AND GENETIC ASPECTS REGARDING PRESYMPTOMATIC TESTING FOR NEURODEGENERATIVE DISEASES

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ETHICAL AND GENETIC ASPECTS REGARDING PRESYMPTOMATIC TESTING FOR NEURODEGENERATIVE DISEASES (Abstract) Neurodegenerative diseases, such as Alzheimer's dementia, Huntington's chorea, Parkinson's disease or spinocerebellar ataxia, manifests into adulthood with an insidious onset, slowly of progressive symptoms. All of these diseases are characterized by presymptomatic stages that preceded with many years of clinical debut. In Parkinson's disease, more than half of the dopaminergic neurons of the black substance are lost before the advent of motor characteristic manifestations. In Huntington's chorea, the progressive neurodegenerative disease could be diagnose prenatal and presymptomatic by analyse of the number of CAG repeats in exon 1 of the huntingtin gene. A similar mechanism represented by expansion of trinucleotide repeats during hereditary transmission from parents to children was identified in fragile X syndrome, spinocerebellar ataxia, spinal muscular and bulbar atrophy, or myotonic dystrophy. Presymptomatic diagnosis in all these progressive diseases raise many ethical issues, due to the psychological impact that can cause the prediction of a disease for which there is currently no curative treatment. Therefore, a positive result can produce serious psychological trauma and major changes in the lifestyle of the individual, instead, a negative result can bring joy and tranquillity. But the problem arises if presymptomatic testing in these neurodegenerative diseases brings greater benefits compared to the possible psychological damage, which can add the risk of stigmatization or discrimination. Keywords: NEURODEGENERATION, DYNAMIC MUTATION, PRESYMPTOMATIC TESTING, ETHICS ASPECTS.

Neurodegenerative diseases (ND) are hereditary or sporadic conditions characterized by progressive nervous system dysfunction, often associated with atrophy of central or peripheral nervous system structures. They are a heterogeneous group of age-associated, chronic illnesses of varying aetiologies, the main challenges for healthcare systems, including informal care and long-term care facilities, in the coming decades (1).

ND had a bad prognosis, after a period of 7–12 years of progressive deterioration of multiple areas of brain, including memory, reasoning, communication and the skills needed to carry out daily activities (2).
The recent discoveries of disease-causing genes in neurodegenerative diseases have generated considerable interest and debate for both physicians and patients regarding routine genetic testing of some ND in the clinic. Molecular genetics provide a powerful tool in the diagnosis of many neurodegenerative diseases and can help better define and classify many of the heterogeneous inherited neurodegenerative disorders. Genetic testing could allow early confirmation of diagnosis and appropriate institution of genetic counselling, but also may provide a genotype-phenotype correlation, select specific patients for clinical drug trials, and offer a better understanding of pathogenesis of the disease (3). Pre-symptomatic testing is usually performed at risk-population and investigate a person with a clear-cut family history, who has no symptoms of ND at the time of testing (4) and looking for genetic mutations that have a high penetrance, such as homozygous or compound heterozygous mutations in recessively inherited genes (e.g. PINK1 and DJ1 genes, in Parkinson's disease) or dominantly inherited mutations (like SNCA or LRRK2 genes, in Parkinson's disease) (5).

Various positions have been advanced regarding the issue of predictive genetic testing for unpreventable or untreatable adult-onset disorders (6). On the basis of a medical benefit argumentation, some have argued that the absence of measures to prevent the disease or its complications or to treat the disease is a reason not to perform this test (7). Although still acknowledging the importance of medical benefit as a justification for predictive genetic testing, it has been recognized that in exceptional circumstances to apply the predictive test could be better than wait that child will arrive in adulthood (8).

Often, the communication of genetic information is a difficult issue and may have a powerful psychosocial impact on the child and its parents. Thus, in the case of a positive test, children could present anxiety or depression, and the parents could present a feeling of guilty. This process could be complicated by several factors. Usually, genetic testing is made only one time during the life and could have important consequences. Thus, the individual needs to think very carefully before deciding to take such a test. In majority of diseases is still not an ideal biomarker able to improve differential diagnosis, track disease progression and measure treatment efficacy and the method could have not conclusive results or be genetically uninformative (7, 8).

However, the feasibility of routine genetic testing of ND in the clinic is still fraught with many unanswered questions. “Which gene must be analysed in a particular case?” “Which clinical features are more adequate to select patients for genetic testing?” “Is the sensitivity and specificity of the genetic test high enough?” “Which are the consequences of a positive or ambiguous result?” “Which will be the patient's and physician's attitudes in case we will discover a genetic abnormality?” (6).

**Genetics of neurodegenerative diseases**

**Alzheimer's disease**

Alzheimer's disease (AD) is the most prevalent ND, accounting 50-70 % of all dementias. In a report of Commission of the European Communities, from 2009 is estimated that 7,300,000 peoples from Europe present AD and probably in 2020 the number will be double (9). The pathogenic mechanisms imply structural and chemical changes in the brain, leading to the death of neurones. For late-onset AD
cases the mechanism implies interaction of multiple genetic and environmental factors. Early-onset (before age 65) AD tends to cluster within families, but represents only 10% of AD (10). Among these, one half is caused by a heritable mutation in gene encoding one of 3 proteins: presenilin-1 (PS1), presenilin -2 (PS2), or amyloid precursor protein (APP). All these mutations are dominant, and the carriers develop AD in nearly 100% of cases. For multifactorial forms the most important gene seems to be the gene of apolipoprotein E (APOE). The gene is located on chromosome 19 and presents three variants: APOE ε2, APOE ε3 and APOE ε4. Only APOE ε4 has been validated, by genetic linkages, as an AD risk factor. The heterozygous for APOE ε4 have a 3-5 time higher risk, while homozygous for APOE ε4 have a 8-18 time higher risk for late-onset AD. However, a genetic test for APOE ε4 has little predictive value (11, 12).

Other genes which are linked to increased risk of AD are CLU, PICALM, CR1, BIN1, ABCA7, MS4A, CD33, EPHA1 and CD2AP. Variants of these genes are linked to significant differences in risk of Alzheimer’s, but their effects are much smaller than for APOE (10-13).

Despite the progresses concerning genetic, behavioural, cardiovascular, and nutritional risk factors, long-term prevention studies have yet to be conducted to see if the incidence of AD can be reduced.

**Parkinson disease**

Parkinson disease (PD) is characterized by a loss of dopaminergic cells in the substantia nigra pars compacta and the presence of Lewy bodies (14). The recent new discoveries of a number of disease-causing genes (such as α-synuclein, parkin, UCHL1, PINK1, DJ-1, LRRK2) in PD have generated considerable interest for presymptomatic genetic testing of PD in the clinic. Early-onset autosomal-recessive PD forms were associated with mutation in PINK1 and DJ-1 (PARK7) genes (15, 16). LRRK2 (PARK8) gene is implied in autosomal-dominant PD with similar phenotype to sporadic PD, but the brain changes generated by LRRK2 mutations are extremely variable. The most common mutation of LRRK2 gene is G2019S substitution present in different populations worldwide. Other possible target genes are PRSS25, UCHL1, synphilin and NURR1 (17). However, the promises and limitations of genetic testing in PD must be confirmed by detailed scientific studies, which will allow also guidelines and recommendations for such genetic testing.

**Huntington disease**

Huntington disease (HD) is a neurodegenerative, autosomal dominant disorder with late-onset, caused by the expansion of a CAG repeat in the first exon of the HD gene, located on chromosome 4p16.3. Usually symptoms begin in 30s or 40s decades by involuntary choreic movements, loss of voluntary control of movement, followed by cognitive impairment and personality disorder tending to depression, anger and temper outbursts (18). The number of CAG repeats is correlated with clinic manifestation of the disease. Normal individuals have 6 – 26 CAGs (class 1) and the allele is stable. In the presence of 27–35 CAGs (class 2) phenotype is normal, but the allele is expandable during spermatogenesis. The individuals with 36–39 CAGs (class 3) have a phenotype with reduced penetrance. The presence of more than 40 CAGs (class 4) is associated with fully penetrated forms (19, 20).

The test for the HD gene is fast, rela-
tively inexpensive, and highly sensitive, with positive and negative predictive values approaching 99 percent. Genetic testing can be performed at any stage of development, even before the onset of symptoms. Many ethical aspects could be identified in the predictive testing of HD, like autonomy, beneficence, non-maleficence, inequality, paternalism or even medical errors. An example of ethical issue could be the case of monozygotic twins, when one brother would to apply the predictive test and other brother do not accept this. In this case is an ethical conflict between autonomy and coercion, but also is necessary to quantify which action is more beneficial: to know if mutation is present or to not know is the mutation is present (21).

Spinocerebellar ataxia diseases

Spinocerebellar ataxias (SCAs) constitute a large group of heterogeneous disorders characterized by a degeneration of the spinal cord and the cerebellum, that cause imbalance, poor coordination, and speech difficulties. Some types of ataxia are inherited, including dominantly inherited diseases and about 30 different gene mutations have been found. Among the more common inherited ataxias are Friedreich’s ataxia and Machado-Joseph disease. Ataxia can also be acquired after stroke, multiple sclerosis, tumours, alcoholism, peripheral neuropathy, metabolic disorders, and vitamin deficiencies. One of the common genetic defects is an expansion of a CAG triplet repeat in genes that regulate the production of different proteins, but the number of CAG repeats varies among the different types of SCAs. Normal individuals with premutation have an increased risk of developing symptoms late in life and also to have abnormal children (22, 23). The correlation between the mutations and the disorders remains unclear, but one explanation may be that the encoded abnormal protein has a toxic effect on neurones, which are successively destroyed. Molecular genetic testing for CAG repeat length is a highly specific and sensitive diagnostic test (24). If the mutation is known in the family, pre-natal diagnosis and pre-implantation diagnosis (PGD) are possible. Genetic testing is available for spinocerebellar ataxia types 1, 2, 3, 5, 6, 7, 8, 10, 12, 13, 14, 17, 28; dentate-rubro-pallidoluysian atrophy (DRPLA); ataxia-telangiectasia (A-T); Friedreich’s ataxia (FRDA); Ataxia with oculomotor apraxia syndrome types 1 and 2; Marinesco-Sjogren Syndrome; Ataxia with Vitamin E deficiency; Fragile X associated tremor/ataxia syndrome (FXTAS); mitochondrial recessive ataxia syndrome (MIRAS); autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS); and Episodic ataxia types 1 and 2 (20, 22-26).

Particularities of genetic testing for a neurodegenerative disease?

In hereditary forms of ND genetic testing can be applied before or after the setup of symptoms. However, the test does not predict the age of setup and the evolution of disease. The major reason for undergoing testing is to find out for the sake of one’s children, or for family planning. However, the use of this test for the minor persons is denied, and the only exception is the presence of symptoms before the age of 18 (27). Most clinical genetic units follow recommended guidelines or programmes, as the waiting process and dealing with the result are very stressful situations, both for the person tested and for the family. The guidelines indicate the need of several meetings with a multidisciplinary team.
(that include a geneticist, a neurologist, a psychiatrist, an ethical counsellor) started by a preliminary meeting when the data from familial history is stored. After this meeting, follow a pretesting meeting (when are discussed the beneficial and the disadvantages of testing) and finally a meeting when are presented the results and are discussed the consequences (positive or negative) of genetic analyse. In the present, the diagnoses of ND are frequently made on clinical grounds, sometimes supported by radiological methods. The application of genetic testing for ND should be reserved to patients who have a family history of ND or those with very young age at onset (28-30).

**Ethical aspects of genetic testing for adult-onset disorders and ethical aspects**

Testing of young people for adult-onset disorders by presymptomatic and predictive genetic testing is the most controversial predictive testing because we have not any treatment that could prevent the onset and progression of the disease (30).

A big problem concerning presymptomatic genetic testing in high risk individuals for ND is related to answer to question - *Do people really want to know the truth?*. Other difficulties are related to collect DNA samples from relatives, who need neurological examination and genetic counselling, problems that rais complex psychological, ethical, legal and moral conundrums (31).

Presymptomatic genetic testing for neurodegenerative diseases generates some ethical conflicts. The authors identified four types of issues. First, in some ND exists juvenile form (for example HD) and this could complicate the decision to make or not the test. Second: in some diseases the preventive measures or the therapies are uncertain. Third: the best interest is interpretable; in some situation the best interest for child is not identical with best interest for family and in these situations the physicians must protect the rights of child in relation with family. And finally, the guidelines must be concordant with local legal or cultural context (32).

Most people have these tests in hopes of negative results, but inevitably, such hopes are often dashed by tests that come up positive. Many other subjects (in particular, those in families with a history of disease) choose to be tested so that, in the event of a positive test, they can make lifestyle changes or pursue clinical interventions that may alter the course of the disease to which they are predisposed. Unfortunately, a positive genetic testing for ND do not allow a preventive or curative treatment like in other diseases (i.e. breast cancer, colorectal cancer) and this could generate psychological trauma. Thus, a positive DNA test for HD or (early-onset) AD means that, if the person lives to middle or late middle age, the disease will manifest, and will be unstoppable once it does. This explains why in generally only about 15-25 percent of people at risk get tested (33).

For some ND, the pathogenic mechanism is linked to a variable amplification of DNA repeats sequences during vertical transmission and generates the phenomenon of anticipation. Also, the severity of the symptoms is correlated with the size of the amplification, but it is difficult to predict severity in a future generation based on mutation size in a parent (34).

Other problem that complicates the genetic testing in ND is the varied penetrance. For example, the penetrance of G2019S mutation associated with Parkin-
son’s disease is highly age dependent, increasing from 17% at age 50 years to 85% at age 70 years (35).

So, in the absence of a possible clinical response, a test confirming or even suggesting future development of a fatal neurodegenerative condition can have a devastating psychological impact on the patient. These risks may include changes in the individual’s perception of self, stresses in relationships with friends or family, discrimination in the workplace or community, difficulties obtaining or keeping insurance, unfavourable adjustment of disability benefits, and other concerns related to privacy and confidentiality. A positive result could be beneficial, because sometime is preferable to known that you have a certain type of mutation in opposition with the anxiety generated by the absence of a clear answer. Also, the result of genetic testing could profoundly influence patient decision making with respect to finances (i.e., providing for inevitable hospice care), family planning (i.e., marriage or reproduction), and fostering personal relationships. Finally, the weighing of risks and benefits of genetic tests is a decidedly personal endeavour (36).

Although, the guidelines for presymptomatic genetic testing in ND vary among countries, some key elements include the following steps: neurologic neuro-psychologic and psychiatric evaluation, genetic counselling, sign informed consent document, psychological assessment, review of the potential impact of the test, disclosure of results in person and arrange post-result follow-up. For the testing will exclude patients with significant psychiatric disorders or those undergoing stressful life circumstances causing emotional upheaval. During the pre-test counselling should be provided the explanations concerning implications of positive and negative results. All DNA determinations must be carried out independently at least twice and the results will be analysed after a genetic linkage computer analysis of haplotypes (the data from relatives must be analyzed anonymously to prevent the transmission of inappropriate information). Usually, the process is long and need many counseling sessions as the patient needs a psychological support. If the patient agrees the idea to inform other members of family about its genetic status, the patient could be accompanied by a relative during a minimum one session and also for the disclosure session when the results will be communicated. The diagnostic information must be given in a face-to-face session, because patient needs to discuss what this information may mean, even if the outcome is genetically uninformative. Long-term follow-up is essential, particularly for those who test positive for the gene. The testing are applied only to persons aged 18 years or older, must be totally voluntary and the results remain totally confidential (9,30-36).

Conclusions

The neurodegenerative diseases represent a group of diseases with late onset, invariable evolution to neurological and cognitive degradation, have not yet any pathogenic therapy and have a genetic component more or less important. In some such conditions the predictive genetic testing before onset of symptoms is possible, but is not available for general population. Such testing may be useful where a family member has already been diagnosed to carry a known mutation.

The genetic testing must be preceded by a genetic counselling respectful of the reli-
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gious and cultural perspectives and traditions of individuals. Genetic counselling is complicated by a multitude of aspects, such as the apparition of a de novo mutation in a negative history family or familial factors, such early parental deaths, adoption, and illegitimacy. Although the medical advances will be inherent and hopefully beneficial to the persons suffering from ND, the psychological and social support by sensitive, timely, and accurate counselling will remain an essential aspect in presymptomatic genetic testing.

REFERENCES

5. Wu-Chou YH, Chen YT, Yeh TH et al., Genetic variants of *SNCA* and *LRRK2* genes are associated with sporadic PD susceptibility: A replication study in a Taiwanese cohort, *Parkinsonism Relat Disord*, 2013, 19 (2): 251-255
9. *** Commission of the European Communities, Brussels, 22 July 2009 - Council recommendation on measures to combat neurodegenerative diseases, in particular Alzheimer's, through joint programming of research activities, http://www.alzheimer-europe.org/Policy-in-Practice2/EU-Action-on-Dementia