

Genetic counselling and testing for Alzheimer's Disease and Frontotemporal Lobar Degeneration: an Italian consensus protocol

Running Title: ItalianDIAfN Protocol for Genetic Counselling

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BACKGROUND Genetic testing of familial Alzheimer's disease (AD) and frontotemporal lobar degeneration (FTLD) is attracting interest thanks to innovative primary prevention clinical trials and increased request for information by at-risk individuals. However, ethical, social and psychological implications are paramount and genetic testing must be supported by structured genetic counselling. In Italy, practice parameters and guidelines for genetic counselling in dementia are not available.

AIM To develop a nationally harmonized protocol for genetic counselling and testing of familial AD and FTLD.

METHODS Activities were carried out in the context of the Italian Dominantly Inherited Alzheimer's and Frontotemporal Network (IT-DIAfN) project, a national network of centres of excellence with expertise in managing patients with familial AD and FTLD. A survey of the literature on genetic counselling protocols and guidelines was conducted. Local protocols for genetic counselling were surveyed. Differences and commonalities among protocols were identified and discussed among project partners. Consensus was reached following implicit aggregation methods.

RESULTS Consensus was reached on a protocol for patients with clinically diagnosed familial AD or FTLD and a distinct protocol for their at-risk relatives. Genetic counselling should be provided by a multidisciplinary team including a geneticist, a neurologist/geriatrician, and a psychologist/psychiatrist, according to the following schedule: (i) initial consultation with tailored information on the genetics of the dementias; (ii) clinical, psychological and cognitive assessment; if deemed appropriate (iii) genetic testing following a structured decision tree for gene mutation search; (iv) genetic testing result disclosure; (v) psychological support follow-up.

CONCLUSIONS This genetic counselling protocol provides Italian centres with a line of shared practice for dealing with the requests for genetic testing for familial AD and FTLD from patients and at-risk relatives, who may also be eligible participants for novel prevention clinical trials.

1. INTRODUCTION

Alzheimer's Disease (AD) and frontotemporal lobar degeneration (FTLD) are two of the most common forms of dementia. Although the majority of cases are sporadic (i.e. without a family history), a markedly familial component has been reported in 60% of early onset (<65 years) AD patients [1] and in 25-50% of FTLD patients [2,3]. An autosomal dominant mode of inheritance is found in about 1-5% of AD and 10-50% of FTLD cases [4,5]: specifically, pathogenic AD mutations were identified in the Amyloid precursor protein (*APP*) [6] and the Presenilin (*PSEN1* and *PSEN2*) genes [7,8], while the genes mainly involved in FTLD are the Microtubule associated protein tau (*MAPT*) [9-11], the Progranulin (*GRN*) [12,13] and Chromosome 9 open reading frame 72 (*C9orf72*) [14,15]. Since the majority of *GRN* pathogenetic mutations cause protein haploinsufficiency, the dosage of circulating progranulin has been proposed as a useful tool for a quick and inexpensive large-scale screening of *GRN* mutations carriers [16-19].

Genetic testing for AD and FTLD is changing rapidly due to the increasing availability of new and faster technologies for DNA test, and it is attracting interest from patients and families thanks to innovative primary prevention clinical trials targeting genetic dementia and to the growing request for information by at-risk individuals. Two recent innovative clinical trials targeting genetic dementia are the Dominantly Inherited Alzheimer Network Trial for AD (DIAN-TU, www.clinicaltrials.gov/show/NCT01760005) and the Presymptomatic Neurodegeneration Initiative for FTLD (PreNI, www.neurodegenerationresearch.eu/initiatives/jpnd-alignment-actions/longitudinal-cohorts/call-for-working-groups/call-results/). Genetic mutation carriers, both symptomatic and asymptomatic, could therefore be eligible participants for these novel clinical trials. Genetic testing in symptomatic patients is used to confirm the clinical diagnosis (diagnostic DNA test); genetic testing in cognitively unimpaired relatives of patients with a mutation identifies those who will develop the disease in the future (predictive DNA test) [20-23]. In at-risk asymptomatic relatives, predictive genetic testing offers the possibility of assessing their personal risk, thus allowing them to organize their lives and make informed career or reproductive choices and decision [23]. Undergoing predictive tests may help at-risk individuals to cope emotionally with their genetic risk by reducing uncertainty about their status and focusing on planning for the future [24], as suggested by a seminal

study in Huntington's disease [25]. As compared to Americans, Italian at-risk relatives expressed higher intentions to undergo genetic testing [20].

However, together with these potential advantages, genetic tests have important ethical, social, legal and psychological implications for the patients and the whole family. In fact, the identification of a genetic mutation in a patient implicitly defines the risk for the other family members, with possible implication for their health and their future. On the other hand, information on genetic status cannot be sensibly used for therapeutic purposes, as no approved disease-modifying treatment is available to date. In healthy at-risk individuals, there are concerns that a positive genetic testing may trigger a negative psychological response, such as severe depression, anxiety, helplessness, or even suicidal ideation [26]. From a social/legal perspective, there may be issues of potential genetic discrimination, difficulties in finding a job or service assistance (including insurance implications), and in creating relationships [21]. Current guidelines for AD and other inherited dementia recommend that genetic testing should be offered within a proper genetic counselling procedure [21]. For asymptomatic at-risk individuals, the protocol established for Huntington's disease and recently revised [27] is recommended.

With the advent of the prevention clinical trials in dementia, such as DIAN-TU for AD in pre-symptomatic mutation carriers, disclosure of genetic status is a pre-condition to participate in a trial in Italy. Such disclosure cannot be made without effective pre- and post-genetic testing support [28].

To date, in Italy no practice standards and consensus-based guidelines are available for genetic counselling of familial AD or FTLN. The IT-DIAfN project, a network of Italian centres of excellence with recognized experience in managing patients with familial AD and FTLN, aimed at developing a harmonized and structured protocol for genetic testing and counselling for those families, both for symptomatic patients and at-risk relatives. As inherited AD and FTLN are genetically heterogeneous and symptoms often overlap, we also generated a decision tree to assist clinicians/researchers in mutations search.

2. METHODS

This study was conducted as part of the "Italian Dominantly Inherited Alzheimer's and Frontotemporal Network (IT-DIAfN)", a national project funded by the Italian Ministry of Health (RF-2010-2319722, Bando Ricerca Finalizzata 2010). It gathers six centres of excellence in the study of genetic AD and FTL: IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli of Brescia, IRCCS Fondazione Istituto Neurologico Carlo Besta of Milan, University of Florence, Centro Regionale di Neurogenetica of Lamezia Terme, University of Brescia, and Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico of Milan. A working group was composed by geneticists, psychologists, neurologists, and bioethicists, with at least one representative from each centre, with the collaboration of the Department of Health Sciences, University of Genova and Division of Medical Genetics, Galliera Hospital, Genova.

As a first step, the group conducted a survey of genetic counselling protocols approved by the local ethics committees and in use at each centre. In particular, the group collected information regarding the professionals involved in the genetic counselling team, tests used for the assessment, phases, procedures, timelines, informed consent forms, access criteria and specific requests from the local ethics committees. Moreover, other specific issues were addressed, such as the informed consent/refusal for storage of the biological samples in a biobank for possible future research studies. Literature search and experience from centres were combined to develop a decision tree to assist in the search of the mutation, to be more accurate, and to reduce time and cost.

A literature search was conducted to analyse guidelines or recommendations available for other genetic diseases, such as Huntington's disease [27,29,30], or in use in other countries. Italian regulations for data protection were considered (The Italian Data Protection Authority, General Authorisation No. 8/2014 for the Processing of Genetic Data, doc. web No. 3632835).

In February-March 2013 the working group participated in the first meetings (one in-person and one *a remoto*, via teleconferences) aimed at identifying and discussing the surveyed differences and commonalities among protocols. The first draft was discussed during an in-person meeting in July 2013 and circulated to the working group in the ensuing months. All comments and feedbacks were periodically summarized and collated by the Project Coordinator, who was in charge of moderating the discussion via electronic communication and of reconciling the different viewpoints to reach a

consensus. The final consensus was reached in October 2014 following implicit aggregation methods [31]: the group experts in each field guided the decision-making process and the final consensus was defined by majority position. Further comments from SINdem (Italian Society for the study of Dementias) were also taken into account.

The harmonized protocol for genetic counselling was approved in February 2015 by the Ethics Committee of the Coordinating Centre of the IT-DIAfN project (IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli of Brescia).

3. RESULTS

3.1 Survey for local protocols

The results of the survey are reported in Table 1. In all centres genetic counselling was provided by a multidisciplinary team, including a geneticist, a neurologist, a psychologist or a psychiatrist. The schedule of the consultation and the procedures varied among centres. Three centres divided the procedure into three consultations: the first was an informational meeting, where the family history was collected and the subjects underwent a neurological and a psychological examination; the second was the blood sample collection, while the third was the disclosure of the genetic test results. The other three centres divided the pre-test phase into two visits: one informational and one for the assessment of the patient. The timetable for the visits and for the supportive follow-up varied from centre to centre. Moreover, some centres considered the follow-up as non mandatory or they offered it only in case of positivity at the test. Different tests were used to assess different domains locally (Table 1). Only two centres had a specific protocol for symptomatic subjects (diagnostic test), while all centres had a protocol for at-risk relatives.

3.2 Harmonized protocol for genetic counselling

3.2.1 Target population

Genetic counselling can be requested (and eventually stopped or suspended at any stage) by (i) symptomatic patients with a positive family history suggestive of an autosomal dominant genetic cause for AD and FTLN and (ii) relatives of patients with proven genetic AD or FTLN. Each subject has to be \geq

18 years old. A positive family history is defined within the group based on the presence of one of the following criteria: i) at least three affected first-degree relatives in two generations, irrespectively of the age at onset; ii) at least two affected first-degree relatives in two generations, with at least one with onset at ≤ 65 years; or iii) one affected family member with onset at ≤ 60 years or with a suggestive clinical phenotype (e.g. dementia with atypical presentation [32], recurring presence in other relatives, peculiar geographic origin).

Cognitively unimpaired at-risk relatives enter the counselling and are eligible to undergo the genetic testing only after the proven presence of the mutation in a first-degree symptomatic relative.

Two specific genetic counselling protocols were defined: one for symptomatic patients (Section 3.2.4) and one for at-risk relatives, with a more relaxed and prolonged schedule (Section 3.2.5).

3.2.2 Genetic Counselling Team

Genetic counselling is provided by a multidisciplinary team of health professionals who work together to provide an individual or a family with current information and supportive counselling about genetic testing. Genetic counselling is structured and guided by a Geneticist and by a Specialized Medical Doctor with specific competences in neurodegenerative diseases. The Geneticist is a specialist in medical genetics with expertise in neurodegenerative diseases, who had education in genetic counselling. The Specialized Medical Doctor (Neurologist, Geriatrician, and/or Psychiatrist) is the clinical contact person for the patient: this role can be also played by the geneticist, but only if he/she is a medical doctor with a consolidated clinical background for the aforementioned diseases. Beside the Geneticist and the Specialized Medical Doctor, the multidisciplinary team is composed by other professionals (i.e. a Psychologist and/or a Psychiatrist), who have expertise in counselling, and have skills at providing ongoing support to individuals with possible socio-psychological consequences associated with the risk of being a mutation carrier.

3.2.3 Evaluation Tests

A common battery of clinical, psychological and cognitive tests was defined for symptomatic patients and their at-risk relatives. Specifically, the battery includes the Mini-Mental State Examination (MMSE)

[33] and the Clinical Dementia Rating Scale (CDR) [34], the Questionnaire on the Health Status - 12 (SF-12) [35] and the World Health Organization Quality of Life (WHOQOL) [36], for the assessment of quality of life and health status; the State Trait Anxiety Inventory (STAI-Y) [37], the Beck Depression Inventory (BDI) [38] and the Hamilton Rating Scale for Depression (HRSD) [39], for anxiety and depression measurement; the Brief COPE (BC) [40], the Resilience Scale for Adult (RSA) [41] and the Health Locus of Control (HLC) [42,43], for the evaluation of the coping style and the locus of controls. Moreover, a depth evaluation of the risk of suicide is recommended, if the subject showed suicidal thoughts or attitudes in the past or at present (item 9 of BDI and/or item 3 of HRSD). One suggested scale is the Beck Hopelessness Scale [44,45]. In addition to these tests, the at-risk relatives are also assessed with the Big Five Questionnaire (BFQ) for personality assessment [46,47].

The subject is assessed at a pre-test phase, to investigate his/her ability to cope with the test result. Except for the MMSE, the CDR and the BFQ, the assessment with the other tests is repeated post-test at different time-points, to monitor the psychological status of the subject, as specified later in the text and in Figure 1A-B.

Based on the profile of the subject, the multidisciplinary team can suggest to postpone the genetic test. The team continues to follow and support the individual as long as it is deemed necessary by the team or requested by the subject.

3.2.4 Protocol for Symptomatic Subjects

The genetic testing for symptomatic subjects has a diagnostic purpose, i.e. to identify the genetic cause of the disease and confirm the diagnosis of AD or FTLN. The procedures are illustrated in Figure 1A and include: at least one pre-test consultation, one meeting for blood withdrawal for the genetic testing, a post-test consultation, where the genetic status is disclosed (for those who wish to know) and then three follow-ups. The number of the consultations can be increased and the timespan relaxed, if required by the patients or if considered appropriate by the team. Genetic counselling for symptomatic patients should be performed in the presence of the caregiver according to patient's will, and the individual's legal guardian if appointed for the individual patient, to help the patient in the decision-making process,

specifically to help in understanding and appreciating the purpose and possible results of the genetic test and in expressing a choice [21, 48].

3.2.4.1 First consultation: Pre-test

During the first informational visit, the whole multidisciplinary team provides general information regarding the familial forms of dementia and details about genetic counselling (i.e. timespan, team roles, aims and procedures), the reliability of the test and the interpretation of the results. The team should explain medical and genetic terms and avoid technical jargon. The implications of both positive and negative test result are discussed, as well as if and how these results will be communicated to other family members. Patients and their families should understand that multiple possible results may be revealed: a causative mutation in one of the genes, no mutation in any of the genes, a variant of unknown significance, or even mutations or variants in more than one genes. Supportive information is given regarding the diagnosis and risk for the genetic disease in the family and its clinical, psychological, sociological and ethical implications, together with the impact on the family members. The specific implications, prospective benefits and risks of undergoing genetic testing are discussed. The aim is to provide as much clear information as possible, and discuss any question or doubt, to allow subjects to take an autonomous and informed decision whether they are willing to undergo genetic counselling and testing and to know their genetic status. The team avoids to influence the decision of the individual and the family, and aims at facilitating their autonomous decision making.

If agreed, the subject, and/or his/her legal guardian on his/her behalf if appropriated, signs the informed consent form for genetic counselling. The caregiver, if present according to patient's wishes, can also sign the informed consent to express agreement [48]. The family health history is collected to construct the family pedigree and, where possible, a complete family history should be collected using a structured questionnaire. Personal clinical phenotype and family history will be used to guide the algorithm for mutation search (see 3.2.6 Flowchart for genetic testing) and to evaluate whether the genetic test is appropriate (as a guide: [49,50]).

Finally, if not too severely impaired, the subject is assessed using the test described in 3.2.3 Evaluation Tests.

After the consultation, the team provides the patient with a written summary or brochure with all the general information discussed in person, to allow their elaboration and comprehension. In case of doubts, a second informational visit can be scheduled.

3.2.4.2 Second consultation: Blood Sample Collection

At the beginning of the second consultation, the whole team evaluates whether all the information provided during the first visit was fully understood, and it clarifies any remaining doubts or new questions that may arise. The results of the assessment (if done) are discussed. If the patient wishes to proceed with the test, the subject, and/or his/her legal guardian on his/her behalf if appropriated, signs the informed consent for genetic testing and blood sample is collected. The caregiver, if present according to patient's wishes, can also sign the informed consent to express agreement. At this stage, the patient can decide whether his/her sample can be stored in a biobank for possible future research studies on genetic forms of dementia or destroyed after the completion of the analyses.

The team informs the patient about the timespan for the completion of molecular genetic analysis (around two months).

3.2.4.3 Third consultation: Post-test

Within two months from the blood sample collection, the results are made available. If the patient still wishes to know, the genetic test results are disclosed and discussed with the patient, and a written report is given. The whole team should assess the individual's understanding of his/her situation and adapt the language to the patient in order to clearly explain the meaning and the implications of a positive, negative, or unclear test result.

The team should support and assist the patient and the family in expressing their emotional reactions to their genetic status. Before leaving the visit, the presence of psychological distress should be informally assessed.

3.2.4.4 Follow-up

After the disclosure of the results, the team offers on-going support and information. Three different time points for the follow-up were defined: after 1, 6 and 12 months from the disclosure. The team collects information about any possible change occurred in the family and in his/her everyday life. The patient is qualitatively assessed about his/her psychological conditions, and the tests described in 3.2.3 Evaluation Tests are administered, whenever possible.

3.2.5 Protocol for At-risk Subjects

The predictive test in genetic counselling aims at confirming whether the genetic mutation identified in the family has been inherited by the at-risk relative, at a stage where the individual does not present any symptom. This protocol arises much more ethical and psychosocial issues than the diagnostic test in symptomatic subjects, because the potential identification as being carrier of an autosomal dominant mutation almost certainly predicts the future development of the disease in an individual currently healthy. Undergoing a predictive test is a highly personal decision that demands extensive counselling. The procedures are illustrated in Figure 1B and detailed below. The schedule mainly differs from the diagnostic test in prescribing at least two pre-test consultations before the genetic testing, and in having a phone call one week after the disclosure of the genetic status.

3.2.5.1 First consultation: Pre-test

As for the symptomatic patients, the first consultation is an informational and supporting visit, when the whole team covers specific issues of the familial forms of dementia, genetic counselling and testing, and the implications of the results (for further details see 3.2.4.1 First consultation: Pre-test). The presence of a support person is encouraged.

If agreed, the subject signs the informed consent form for genetic counselling. Then, there is an evaluation of the personal and familial medical history.

After the consultation, the team provides the subject with a written summary or brochure with all the general information discussed in person, to allow their elaboration and comprehension.

3.2.5.2 Second consultation: Pre-test

After one month from the first visit, the second one is scheduled, to further discuss the motivation for the predictive test and clarify any doubts which may have arisen.

The subject is assessed using the tests described in 3.2.3 Evaluation Tests.

3.2.5.3 Third consultation: Blood Sample Collection

The procedures are the same as for the symptomatic protocol (see 3.2.4.2 Second consultation: Blood Sample Collection).

3.2.5.4 Fourth consultation: Post-test

The procedures are the same as for the symptomatic protocol (see 3.2.4.3 Third consultation: Post-test).

3.2.5.5 Follow-up

One week after the disclosure of the results, the team (specifically the Psychologist and/or Psychiatrist), contacts the subject by phone and offers on-going support and information. The other follow-up visits are the same as for the symptomatic protocol (see 3.2.4.4 Follow-up).

3.2.6 Flowchart for genetic testing

A decision tree was developed to assist in the search of the mutation in symptomatic patients (Figure 2).

If the patient has recently undergone a lumbar puncture and A β and tau levels in the cerebrospinal fluid (CSF) have been analyzed, this measure guides the genetic screening. If the CSF tau and A β 42 levels are abnormal (i.e. tau/A β 42 > 0.52) [51], genetic mutations linked to AD are searched as first. To determine which gene should be sequenced first (i.e., *APP*, *PSEN1*, or *PSEN2*), patient's age at onset (AAO) is considered: if the patient has an early age at onset (≤ 65 years), *APP* gene (exons 16-17) is sequenced; if negative: all *PSEN1* exons and flanking regions; if negative: all *PSEN2* exons and flanking regions. Instead, if the patient has a late age at onset, *PSEN2* gene is the first choice for sequencing, and then *APP* and *PSEN1*. If all AD genes are mutation-negative, but there is a strong evidence for an autosomal dominant pattern, then genes linked to FTLD are screened. Plasma progranulin dosage for *GRN* null

mutation screening has the priority: if the level is <61.55 ng/ml [19], the presence of the common Italian p.L271fs (exon8:c.811_814del, ref sequence NM_002087) mutation is assessed [52]; if no mutation is found, all other exons are sequenced. Conversely, if the progranulin level is not suggestive for the presence of null mutations (i.e. >61.55 ng/ml), the clinical phenotype is taken into account. *C9orf72* genetic screening should have the priority in patients with FTLD and Motor Neuron Disease (FTD-MND), behavioural variant frontotemporal dementia (bvFTD) with psychosis, or FTLD with cerebellar features, while *MAPT* gene (exons 1, 9-13, as first) should be the first choice in patients with bvFTD and semantic dementia, progressive supranuclear palsy (PSP), corticobasal syndrome (CBS) or an early age at onset (AAO<60 years). When both *C9orf72* and *MAPT* resulted negative, then *GRN* should be tested, given possible false negative in progranulin plasma screening.

When CSF tau and A β levels are normal or not available, the algorithm is based on the progranulin dosage, as described previously. When patient's level of progranulin is normal, the clinical phenotype is taken into account for guiding the analysis. If the clinical phenotype is FTD-like, then the priority between *C9orf72* and *MAPT* is defined as previously described. If the clinical phenotype is consistent with AD, then, according to the age at onset, *APP*, *PSEN1* and *PSEN2* are screened as described previously. When no mutation is found, the other branch in the diagram is followed (Figure 2).

Lastly, if the DNA analysis is done in an at-risk subject, only the mutation found in the symptomatic relative is searched for.

4. DISCUSSION

Genetic testing has ethical, social, legal and psychological implications for patients and their family: when handled by an experienced and qualified multidisciplinary team following structured criteria, these issues can be faced serenely and confidently by families. Here, we present a standard and structured protocol for genetic testing and counselling for symptomatic and at-risk individuals belonging to families with suspected forms of autosomal dominant AD or FTLD. This protocol is not designed for genetic counseling of people who carry susceptibility genetic risk for AD, such as the Apolipoprotein E e4 allele, as this is only a risk factor for dementia and no definitive prediction can be made about dementia in these subjects. Moreover, based on the current knowledge, we would not

recommend genetic counselling to all sporadic FTD cases as the likelihood of finding a mutation in cases with a negative family history is presumably low [49] and would thus unnecessarily increase burden to patients and their families. The IT-DIAfN protocol provides guidance on the schedule, the procedures, the tests and the professional figures involved in genetic counselling, with the aim to support and take responsible care of these families. The tests for clinical, cognitive and personality assessment were accurately chosen to obtain a concise but accurate profile of the individual, helping in personalizing and adapting the support within the counselling to his/her possible reactions.

We have taken into account the guidelines for the genetic testing of Huntington's disease [27,29,30], which is considered the gold standard for genetic testing for adult onset diseases. As recommended by these guidelines, we used a multidisciplinary approach to facilitate the subject's autonomous decision making: the Geneticist as the expert in genetic counselling for AD and FTLN, the Specialized Medical Doctor as the clinical contact person of the patients; and the Psychologist/Psychiatrist as the contact person for counselling, psychological appraisal and support. The schedule and timespan of the visits are similar to those defined for Huntington's disease: several sessions and phases (pre-, post-testing and follow-ups) are crucial to allow free choice, without rushing into genetic testing before all the necessary explanations and information are given. Other recommendations prescribed in the Huntington's disease guidelines were adopted here: i) disclosure of the genetic status is provided both orally and through a written report; ii) availability of the test only to individuals who have come of age; iii) possibility of storing the biological samples in a biobank for research studies after signing the informed consent.

As described in other existent counselling protocols for AD and FTLN [21,22], we recommended that i) the legal guardian of the symptomatic patient is present during the counselling to help her/him in understanding its purpose and possible results of the genetic test and in expressing a choice, and that ii) a symptomatic family member should be tested before an at-risk individual.

The guidelines for genetic counselling for AD defined by the American College of Medical Genetics and the National Society of Genetic Counsellors [21] specified that genetic counselling could be done in-person or through videoconferences. The Huntington's disease guidelines clearly stated that the results of the test should be revealed in-person, and not by phone or mail [27,30], while psychosocial support

should be available close to the person's community, by phone or telemedicine where necessary. A few pilot projects demonstrated that telemedicine can be used to offer medical genetics service on a remote basis [53]. Preliminary evidence suggests that genetic counselling can be effectively delivered in underserved areas, also in late-onset genetic disorders such as familial cancer [54]. The advent of telegenetics could allow the implementation of this genetic counselling protocol in clinical research centres without lowering the clinical standard; it may also facilitate the enrolment in clinical trials of individuals who could not access to tertiary care genetics centres. Nevertheless, it is recommended that the Psychologist/Psychiatrist should be always present in person during the visits.

We developed two specific paths, one for symptomatic patients and one for asymptomatic at-risk subjects, being aware of the difference of the two scenarios. In fact, in symptomatic subjects, genetic testing is aimed at confirming the clinical diagnosis and the genetic cause of the disease. A positive result can provoke hopelessness and sense of guilty for the possibility of transmission of the mutation to the offspring. In the case of asymptomatic subjects psychological, social and ethical issues are even more critical, especially in the absence of prevention or treatment options: a correct and careful counselling will offer them the option of knowing their condition, cope positively with their future and eventually they might be offered the possibility to enter a novel prevention clinical trial. It must be underlined that a negative result has different meanings according to the two different scenarios: in a symptomatic subject, this could not exclude definitely that the disease has a genetic cause (thus it is sporadic), because an unknown/new mutation could be present but not yet been discovered; in an at-risk relative, a negative result confirms almost with absolute certainty that he/she has not inherited the causative mutation found in the affected relative and thus he/she has the same risk of the general population to develop the disease in the future.

Considering the results reported in Binetti et al., 2006 [20], Italian families with familial dementia showed poor knowledge of the disease and poor awareness of personal risk of developing dementia during lifetime. This means that the first consultation should address these aspects in details, guiding families to a free and informed choice.

We developed a step-by-step algorithm for guiding genetic screening on the basis of biomarker results and clinical data, to reduce time and cost of the laboratory analysis. Specifically, the decision tree was defined according to (i) the observed frequency of genes in specific clinical phenotypes in the literature [49,55-58], and specifically in Italian clinical series [52,59-65], (ii) the direct experience of the Italian centres, and (iii) practical considerations, e.g. the high speed and low cost of a given screening procedure (i.e. plasma progranulin dosage). In the future, other biomarkers can be taken into account and implemented within the flowchart, such as the amyloid PET.

The research protocol reported herein reflects the current practice of Italian centres taking part to the IT-DIAfN initiative. Should important innovations be introduced in the clinical practice as well as in laboratory analyses, the protocol will be amended. Following the wide availability of next generation sequencing (NGS) panels for the molecular genetics analysis, the decision tree is subject to changes accordingly. NGS is not currently applied in all centers participating into the IT-DIAfN framework, but appears as the upcoming technology for the mutation analysis in heterogeneous genetic disorders such as AD and FTLT. To date, the NGS approach provides the simultaneous analysis of a panel of 17 genes (*PRNP*, *PSEN1*, *PSEN2*, *APP*, *GRN*, *MAPT*, *TREM2*, *CHMP2B*, *CSF1R*, *FUS*, *ITM2B*, *NOTCH3*, *SERPIN1*, *TARDBP*, *TYROBP*, *VCP*, *SQSTM1*). Other experimental panels of additional genes and SNP polymorphisms are currently under development within the IT-DIAfN group. The mutation search should be completed with two PCR analyses of the *C9orf72* hexanucleotide repeat expansion and the octapeptide repeat region of *PRNP* [66].

This protocol, developed by experts in the field, provides Italian centres with a line of shared practice for offering genetic counselling to eligible individuals who may benefit from genetic testing for familial AD and FTLT, and recruit them through a procedure which is compliant with International recommendations and good practices.

Future steps will be the validation of the harmonized protocol of genetic counselling. This will be performed in two steps: i) after approval by local ethics committees, the protocol will be implemented in all centres participating into the IT-DIAfN project; ii) data from each centre will be collected and the results from the pilot stage will be critically reviewed by the working group who developed the protocol.

The genetic counselling protocol will also be disseminated through medical societies and proposals for amendments will be acknowledged.

We are aware that the entire procedure, including counselling, repeated assessments and testing, is highly demanding, especially in terms of human resources. The procedure is expected to be accomplished within a research environment by a multidisciplinary team in centres with specific expertise – as such it was not designed to be applied in routine clinical practice in its current version. In the light of the suggestion raised during the validation phase, an optimized protocol could be eventually transferred to clinics, provided that evidence of clinical utility has been produced and a health technology assessment has supported its implementation in clinical practice.

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Table 1. Survey of the genetic counseling protocols in use at the local centres.

| | IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli Brescia | IRCCS Fondazione Istituto Neurologico Carlo Besta Milano | Spedali Civili/ Università di Brescia | Università di Firenze | Centro Regionale Neurogenetica Lamezia Terme | Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico Milano |
|----------------------------------|--|--|--|---|--|--|
| | 3 Consultations (+ follow up) | 3 Consultations (+ follow up) | 4 Consultations (+ follow up) | 4 Consultations (+ follow up) | 4 Consultations (+ follow up) | 3 Consultations (+ follow up) |
| Scheduling and phases | 1- Informational meeting, Family health history collection, psychosocial assessment, neurological examination, | 1- Informational meeting, Family health history collection, psychological questionnaires, neurological examination | 1- Informational meeting, Family health history collection | 1- Family health history collection, neuropsychological assessment, neurological examination and informational meeting on the disease | 1- Family health history collection, neuropsychological and personality assessment, neurological examination | 1- Informational meeting, Family health history collection, psychological questionnaires, neurological examination |
| | | | 2- Psychological assessment, neuropsychological assessment, neurological examination | 2- Informational meeting on the genetic risk and the genetic test | 2- Informational meeting | |
| | 2- Blood sample collection | 2- Blood sample collection | 3- Blood sample collection | 3- Blood sample collection | 3- Blood sample collection | 2- Blood sample collection |
| | 3- Disclosure of genetic test result | 3- Disclosure of genetic test result | 4- Disclosure of genetic test result | 4- Disclosure of genetic test result | 4- Disclosure of genetic test result | 3- Disclosure of genetic test result |

| | | | | | | |
|--|---|---|---|--|---|---|
| Professionals in the Team | Neurologist, Geneticist, Psychiatrist, Psychologist | Neurologist, Geneticist, Psychiatrist, Psychologist | Neurologist, Geneticist, Psychiatrist, Psychologist | Neurologist, Geneticist, Psychiatrist, Psychologist | Neurologist, Geneticist, Psychiatrist, Psychologist | Neurologist, Geneticist, Psychiatrist, Psychologist |
| Timetable | 60 days required for the genetic test | Not defined | Not defined | About 1 month between first and second consultation; about 1 month between third and fourth consultation | Blood sample is taken within 4 weeks from the second consultation | Not defined |
| Follow up | At 12 and 24 months (irrespective of test result) | At 6/12 months (not mandatory) | At 15 and 30 days (in case of positivity at the test) | At 1, 3, 6 and 12 months (irrespective of test result) | At 1, 3, 6 and 12 months (irrespective of test result) | At 6/12 months (not mandatory) |
| Clinical/ Psychological, Cognitive and Personality Test | BDI, STAI-Y, BFQ, GHQ, ISS, HLC, SPQ, SLEQ, WHOQOL-100, SDABE | Psychological self-administered Questionnaires | Semi-structured interview, MMPI-2, STAI, ZUNG, Neuropsychological tests | Neuropsychological assessment | MMPI, Mental Deterioration Battery | Psychological self-administered Questionnaires |
| Protocols | Diagnostic and Predictive protocol | Predictive protocol | Diagnostic and Predictive protocol | Predictive protocol | Predictive protocol | Predictive protocol |

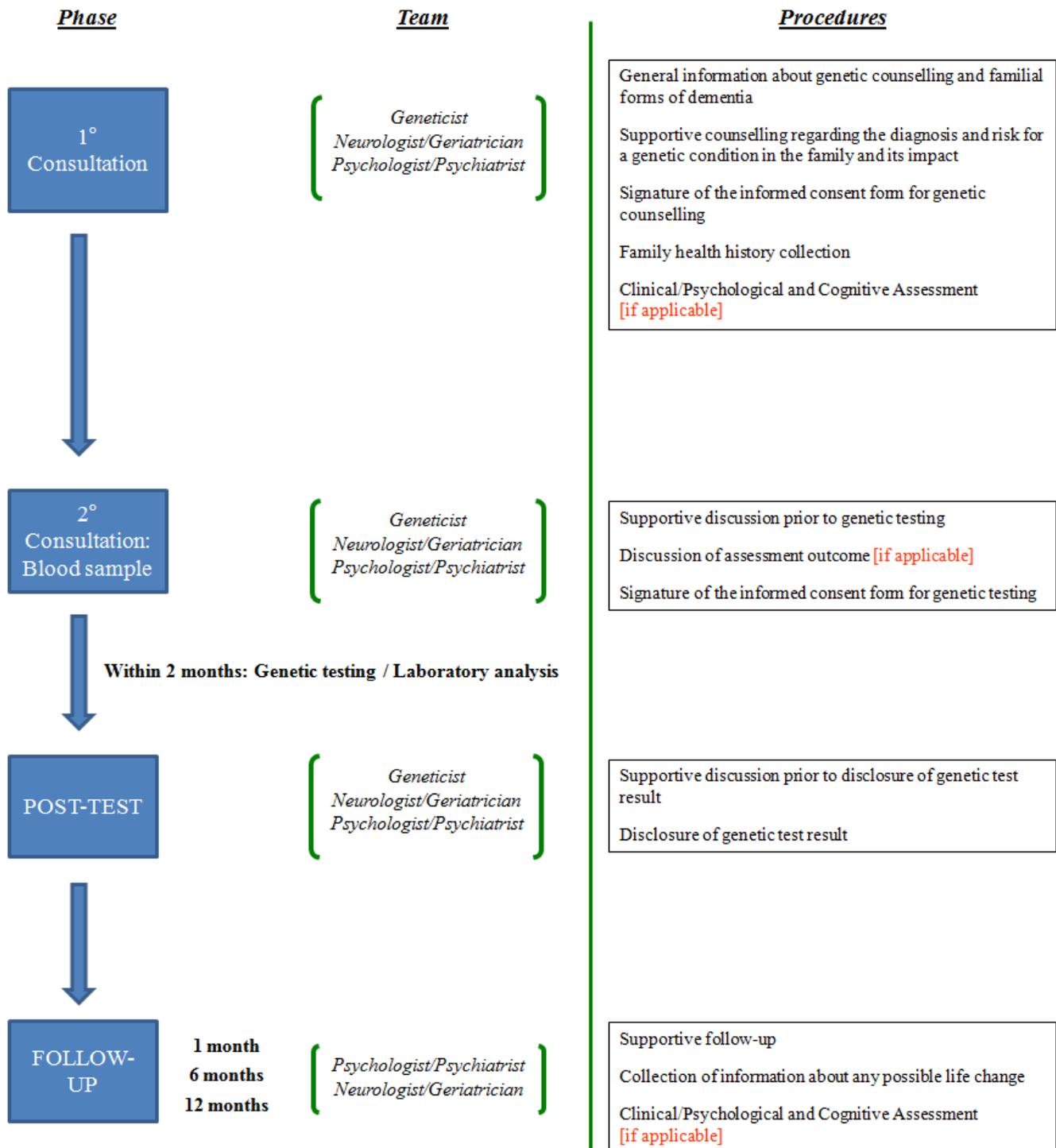
Abbreviations: BDI, Beck Depression Inventory [38]; STAI-Y, State Trait Anxiety Inventory [37]; BFQ, Big Five Questionnaire [46,47]; GHQ, General Health Questionnaire [67]; ISS, Information Seeking Style [68]; HLC, Health Locus of Control [42]; SPQ, Social Problem Questionnaire [69]; SLEQ, Stressful Life Events Questionnaire [709]; WHOQOL-100, The World Health Organization Quality of Life assessment [71]; SDABE, Dementia Attitudes

Beliefs and Experiences [20, 72,73]; MMPI-2, Minnesota Multiphasic Personality Inventory-2 [74]; STAI, State Trait Anxiety Inventory [75]; ZUNG, Zung self-rating depression and anxiety scales [76,77]; Minnesota Multiphasic Personality Inventory [78]; Mental Deterioration Battery [79].

Figure 1. Genetic Counselling protocol. Flowchart for symptomatic subjects (A) and for at-risk relatives (B).

A)

Flowchart – Symptomatic subject



B)

Flowchart – at-risk relative

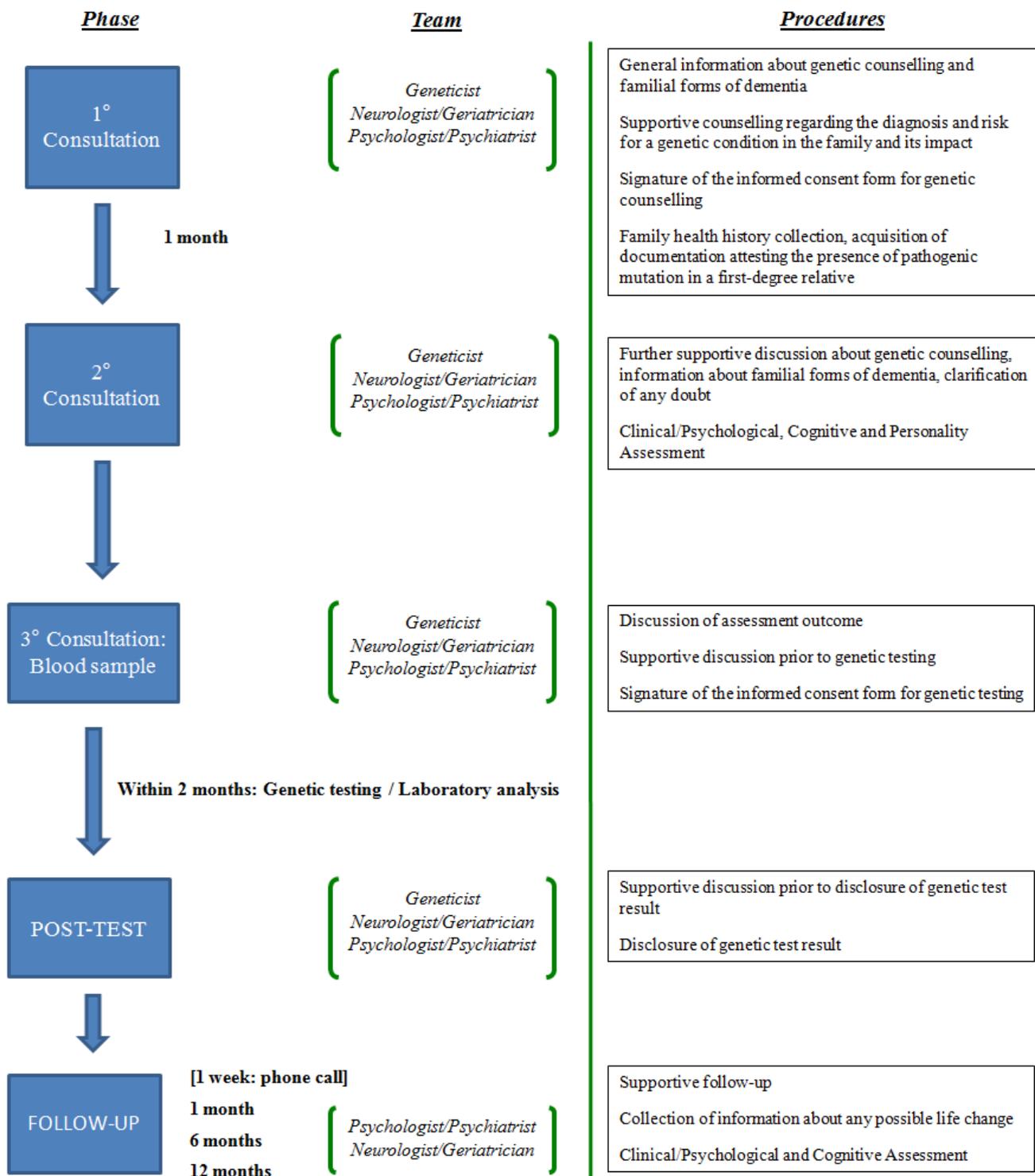


Figure 2. Genetic Testing Flowchart. Step-by-step algorithm to assist in the search of genetic mutations, for cases with abnormal A β and tau levels in the cerebrospinal fluid (CSF) (left panel) and with normal CSF values or data not available (right panel).

