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THE GENETIC BASIS OF PRIMARY ADULT-ONSET DYSTONIA

TUTOR

Professor

Lucio Santoro

PhD STUDENT

Doctor

Marcello Esposito

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ABSTRACT

Objectives. To identify novel genetic loci associated with primary adult-onset dystonia (AOD) and assess candidate genes from the literature.

Secondary aimes. Epidemiological studies: to identify the rate of family history in people affeceted by AOD in Italy and to assess the impact of enviromental factors and clinical features between sporadic and familial cases.

Background/rationale. Primary AOD has variable clinical expression as blepharospasm, oromandibular dystonia, cervical dystonia, laryngeal dystonia and arm dystonia. It usually starts after the age of twenty, has a peak age incidence between the 5th and the 7th decade, and predominates in women. Prevalence estimates vary widely across studies, but crude rates between 430 and 2250 cases per million are probably close to the true prevalence of the condition. AOD can be highly disabling and carry social and financial costs. Primary adult-onset dystonia is assumed to be partly genetic due to its aggregation within families and the identification of specific genetic loci including DYT 1, 2, 4, 6, 7, 13, 16, 17. Linkage to

these loci is rare and accounts only for a small fraction of patients with adult-onset dystonia. Hence the gene(s) that lend risk to commonly occurring adult-onset primary dystonia are not known.

Description of the project. The present project combines the clinical and genetic expertise of 9 leading Italian movement disorders centers. Data were collected from a wide, representative Italian sample of patients with AOD and their first degree relatives. Genetic factors will be studied by the affected sibling pair method (ASP) that allows identification of novel disease associated-loci, and also offers the potential to study candidate gene from the literature. .

Key words: dystonia, linkage analysis, polimorphisms

INTRODUCTION

Primary late-onset dystonia, the most common form of dystonia, can manifest in focal or segmental forms mainly involving cranial, cervical, and upper limb muscles. The various phenotypes of late-onset dystonia may differ for sex distribution, age of onset, and tendency to spread. Nevertheless, there is evidence suggesting that the various clinical forms may share etiologic factors, but information on the etiology of primary late-onset dystonia is limited. A likely hypothesis is that it is a multifactorial condition, in which several genes, along with environmental factors, combine to reach the threshold of disease. Primary adult-onset dystonia [1] is assumed to be partly genetic due to its aggregation within families and the identification of specific genetic loci including DYT1, DYT6, DYT7, and DYT13 [2]. The phenotypic heterogeneity of dystonia within families (6, 10, 11) could suggest that susceptibility factors may lead to a spectrum of clinical manifestations including blepharospasm, oromandibular dystonia, cervical dystonia, laryngeal dystonia or arm dystonia [3]. Linkage to DYT1, DYT6, DYT7, and DYT13 loci is rare and accounts only for a small fraction of patients with adult-onset dystonia [4,5]. A few candidate gene studies using case-control design failed to find convincing evidence

for a role of DYT1 polymorphisms, dopamine receptor genes polymorphisms, the human-leucocyte antigen–DRB locus, and polymorphisms in the homocysteine metabolism in the pathogenesis of adult-onset dystonia. Other candidate genes related to the mechanisms potentially underlying primary dystonia have not been investigated so far. Since the genes lending risk to primary late onset dystonia are not known, support for a genetic contribution comes mainly from family based studies demonstrating unequivocally that, regardless of phenotypic appearance, primary late-onset dystonia can aggregate in families. Although in a few large families an inheritance pattern compatible with an autosomal dominant trait and reduced penetrance is apparent, in most case no more than one or two affected first-degree relative can be detected and inheritance does not appear to be mendelian. Environmental factors influences on pathophysiology of dystonia comes from a limited number of controlled studies. The most consistent findings were the association of diseases of the anterior segment of the eye to blepharospasm (supported by three controlled studies), and the inverse association between coffee drinking and cranial dystonia [6]. No more than one controlled study gave support to other potential environmental risk factors such as scoliosis [7] and neck trauma [8] for cervical dystonia, upper respiratory tract infection for laryngeal dystonia, and working activities that require repetitive and accurate motor tasks for hand dystonia. Overall prior studies left a number

of issues open. First, the rate of family history of dystonia is not precisely known since it varied widely across studies, from 2.5 to 30% [9]. Second, information on environmental risk factors possibly leading to primary late-onset dystonia comes from case-unrelated control studies and it remains unknown whether putative environmental risk factors for dystonia are more frequent among case patients than among their at risk relatives. No family-based case-control study relied on environmental risk factors for primary late-onset dystonia. This is a well established approach to test candidate genetic risk factors as a method to circumvent possible population stratification bias, but it has also been applied to the study of environmental risk factors for Parkinson's disease [10]. Despite affected and unaffected family members are more likely to be overmatched for environmental exposures and life styles than unmatched case and control groups, detecting significant associations with environmental factors in family-based series may help increasing the solidity of the results from case-unrelated control series and identifying covariates to consider in genetic studies. Third, genetic inheritance may explain, at least in part, the etiology of familial late-onset primary dystonia, whereas the role of genes is less clear in sporadic cases for whom a genetic contribution has been suspected but never proved. Likewise, it remains unclear whether environmental factors differently concur to familial and sporadic forms. Given that the genetic basis of commonly occurring familial late-onset

dystonia are not known, one way to assess whether familial and sporadic late onset dystonia are different entities or not might be to check the two groups for relevant demographic/clinical features (including age of dystonia onset, gender and tendency to spread) and environmental risk/protective factors. If familial and sporadic cases have different etiology or a different burden of shared genetic/environmental factors, then different clinical features and differences in the distribution of certain environmental risk factors may be expected between the two groups. Prior family based studies did not approach, or failed to clarify, these issues because they were based on small sample sizes, relied on family history data from proband's interview, a method characterized by low sensitivity in detecting affected relatives as compared to direct examination of at risk relatives, or assigned diagnosis of dystonia to patients with subtle motor signs but no definite dystonia, thus proving to be unreliable or lacking validity.

Accurate identification of patients who have family history of dystonia and those who do not is the first step to perform valid family–based studies. To this aim, clinical examination of most at risk relatives is needed but such method is too expensive and time consuming. Recently a two-step procedure was developed yielding 95% sensitivity and 100% specificity in diagnosing dystonia among first-degree relatives of probands with primary adult-onset dystonia, allowing easier screening of large samples [11]. We

therefore used this procedure to screen first-degree relatives of probands with primary adult-onset dystonia diagnosed according to stringent criteria from a large, multicenter Italian series. The main aim of this project is to investigate the genetic basis of primary adult-onset dystonia with particular reference to the identification of novel disease-associated loci and the evaluation of the association between candidate genes from the literature and primary adult-onset dystonia. As it has been stated, the gene(s) that lend risk to commonly occurring adult-onset primary dystonia are not known. A few loci (accounting only for a small fraction of patients with adult-onset dystonia) were identified by linkage analysis in some large multigenerational families. Yet such families are difficult to find, owing to the late onset of the condition and the inheritance pattern possibly consistent with an autosomal dominant trait and reduced penetrance or, alternatively, with a polygenic transmission [2,3]. As an alternative to linkage analysis in large families, model free non parametric methods can allow identification of novel genes in complex diseases [12]. Tools for identifying novel loci include the affected sib-pair (ASP) method which is based on excess sharing of alleles among affected siblings over the general population [13]. No published study has explored the applicability of the ASP approaches in the field of adult-onset dystonia, whereas these designs have proven useful in the search for susceptibility loci involved in other complex diseases such as Parkinson's disease [14] and Alzheimer's

disease. Moreover the present project investigated whether polymorphisms of neurotrophic factors controlling plasticity mechanisms, e.g. BDNF; there may be a relationship between the aforementioned factors and the mechanisms underlying primary adult onset dystonia. BDNF can positively modulates brain plasticity mechanisms that are probably altered in primary adult-onset dystonia. Evidence from studies in animal models (rat hippocampus) shows that BDNF selectively potentiates glutamatergic transmission and, consensually inhibits GABAergic transmission [15]. The is involved in intracortical inhibition phenomena that seem to be impaired in patients with primary adult-onset dystonia [16]. Given the prevalence of primary adult-onset dystonia to reach the proposed objectives in a reasonable time necessarily means conducting a study based on a population of prevalent case patients recruited at Italian leading movement disorders centers. The critical point inherent to the project is the recruitment of an adequate number of affected sibling pairs to have sufficient power to detect novel susceptibility loci strongly associated with the disease : 100 to 200 ASPs would probably be required under the hypothesis of a few major genes being responsible for a large proportion of disease risk (details discussed in genetic study methods). Collection of genetic data from a large number of ASPs from different Italian areas (including South, Center, and Northern Italy) would allow us to take into account and examine potential ethnic, geographic, gender, and clinical

differences in respect to genetic risk factors for primary adult-onset dystonia. Identification of genetic loci associated with primary adult-onset dystonia would allow new insights into the pathophysiology of the disease, and would facilitate diagnosis, genetic counseling, and development of new treatment options with health gains and benefit for families and public health system.

Secondary aims of this project were to identify the rate of family history in people affeceted by AOD in Italy analysing the large sample of patients included in the project and to assess the impact of enviromental factors (eye diseases and coffee drinking) and clinical features features (including age of dystonia onset, gender and tendency to spread) between sporadic and familial cases.To get this piece of information has implication for designing the best strategy aiming at the identication of the etiologic basis of primary late-onset dystonia.

METHODS

Probands were identified during a 12 months study period among consecutive outpatients attending nine Italian tertiary referral centers for movement disorders and living no more than three hours travelling distance from the centre. The ethical committees of each participating clinical centers approved the study. Inclusion criteria were a diagnosis of dystonia according to published criteria (slow dystonic movements and definitely abnormal postures occurring at rest or activated by specific tasks); age at first symptoms > 20 years; duration of disease greater than one year; and no other neurological abnormality except tremor associated with dystonia. Exclusion criteria were features suggesting secondary dystonia—dystonia plus and heredodegenerative dystonia—and specific referral for familial dystonia.

Family study

First-degree relatives were screened for familial aggregation of dystonia by a two-step procedure as reported [11]. Briefly, relatives received first a self-administered diagnostic questionnaire that they filled in if they agree to participate into the study; thereafter, relatives screening positive on the questionnaire and relatives screening negative who had low education (< 8 years of schooling) were examined at their homes by a trained physician

who used a standardised protocol described elsewhere, including triggering manoeuvres for dystonic movements or postures in apparently asymptomatic subjects. Performing relatives' examinations at their home gave us the opportunity to also examine a certain number of relatives who should not have been examined, that is relatives screening negative at the questionnaire who had > 8 years of schooling. Diagnosis of dystonia was not assigned to excessive blinking with no evidence of the Charcot's sign [17], isolated apraxia of eyelid opening, unusual tight hand gripping with writing or other manual activities, shoulder elevation without significant limitation of shoulder movement, or isolated head/upper limb tremor with no signs of dystonia. We evaluated the agreement of the diagnosis of dystonia at different body sites among the examiners from the participating centers by k statistics using viderecordings from 20 patients with late-onset dystonia, 10 patients with movement disorders other than dystonia, and 10 healthy controls. According to the Landis classification, substantial (k index between 0.6 and 0.8) to almost perfect (k > 0.8) interobserver agreement was obtained for the diagnosis of BSP(k=0.81) , OMD (k= 0.71), CD (k = 0.82), laryngeal dystonia (k =0.73) and FHD (k=0.75) [18].Information from probands and family members was collected about age, sex, education (years of schooling), age at the onset of dystonia (defined as time of first symptoms), duration of disease (time elapsing from

age at first symptoms to age at investigation), focal dystonia at presentation and time to spread when appropriate.

Risk factor study

All Probands and the family members who were directly examined underwent a standardized interview on environmental risk factors (coffee drinking and diseases of the anterior segment of the eye) administered face-to-face by a trained medical interviewer for each centre. Assessors were not blinded to the affected/unaffected status because it is difficult to blind a patient with dystonia from a normal subject but they were unaware of the study hypothesis. Non-decaffeinated coffee intake was assessed by asking the subject ”have you ever drunk non-decaffeinated coffee”? Participants who responded “yes” were asked whether they began and, if applicable, quit drinking coffee before the main reference age [19]. This was the age of onset of dystonia affected case patients. The main reference age of unaffected family members was calculated by subtracting the number of years corresponding to the average disease duration of the case relative to their actual age. According to the status at the reference age, participants were classified as never, ex- or current coffee drinkers. In a test-retest repeatability study previously performed by our group, self-reported data on coffee drinking status at the main reference age showed high repeatability as assessed by k statistics[6]. The questionnaire section

assessing ophthalmological complaints has been previoulsly described and validated. Briefly, to be classified as presenting ocular symptoms, subjects had to provide an affirmative answer to at least one of four questions: Do your eyes ever feel dry? Do you ever have a gritty or sandy sensation in your eyes? Do your eyes ever have a burning sensation? Are your eyes ever red? Subjects who responded "yes" were asked about the year in which these problems began and the year, if ever, in which they remitted. The questionnaire had 76.5% sensitivity, 94.1% specificity, 92.8% PPV (positive predicitve value), and 80% NPV (negative predictive value) in diagnosing disorders of the anterior segment of the eye in both patients with cranial dystonia and control subjects.

Statistical analysis

Statistical analysis was performed using Stata11 package. Unless otherwise specified all data are expressed as means ± SD. Differences between groups were examined using the chi-square test and a one-way ANOVA with Newman-Keuls post hoc test. The relationships of coffee intake before the reference age and prior eye diseases to case/control status (affected / unaffected family members) was estimated by means of logistic regression models with unequal case/control ratio, and odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Sex was represented in the

model by a categorical variable (1 if woman, 0 if man), with age and years of schooling as continuous variables. Case-control subjects for whom information was missing were excluded from the analysis. The relationship between family history of dystonia and spread was estimated by Kaplan-Meier survival curves and Cox proportional hazard regression models. For both methods the endpoint was time to spread. Patients without spread were included in the survival functions for as long as the length of observation, and their data were censored beyond that time. Patients with focal cranial dystonia and duration of disease <5 years and patients with focal extracranial dystonia and duration of disease <10 years were excluded from spread analysis. Statistical power was assessed according to Parmar and Marchin. For all statistical methods, p values < 0.05 were considered to be significant.

Genetic study

Linkage analysis for the identification of new genes

Identified affected sibling pairs will have a blood sample taken for DNA testing. The ASP mapping method was used for linkage analysis of the collected affected sib-pairs. Genome wide scan was conducted using 400 markers with an average distance of 5-10 cM. The data were checked for typing inconsistent with Mendelian transmission or with family structure using PedChec [20] .DNA at the same locus on two homologous

chromosomes is said to be identical by descent (IBD) if it originated from the same ancestral chromosome. If two homologous chromosomes from different people are IBD at some locus, the people are related. If two homologous chromosomes from the same person are IBD at some locus, this person is inbred, i.e. has related parents. Two people, neither of whom is inbred, can share DNA IBD at a particular locus on either 0, 1, or 2 chromosomes.[21]. For the purposes of the ASP method, the genetic contribution is measured in terms of the sibling risk ratio (lambda), that is the ratio of the disease prevalence in siblings to the disease prevalence in the general population [22,23]. The overall lambda can result from the contribution of *n* loci, where n is the researcher's best guess of the number of loci that make an important contribution to the risk of disease. If the n loci act in a multiplicative manner and their contributions are equal, then the locus specific lambda is expressed by the *n*th root of the overall lambda. From the above, the proportion of sibpairs identical by descent (IBD) at the locus of interest could be calculated by the following formula: IBD = ¼ (3 lambda –1) / lambda. In the case of a marker being unlinked to the disease we would expect allele sharing between affected siblings to be 50% (IBD = 0.5). If the marker is linked to the disease, the probability of an affected sib pair sharing IBD alleles will be higher than 0.5. Sample size can be estimated based on standard tests comparing a proportion with a known proportion, and assuming 80% power to detect linkage at a

significance level of 0.0001. The sample size estimates resulting from use of different sibling risk ratio (lambda) (Tab 1); owing to uncertainties in the prevalence of primary adult-onset dystonia , in fact, lambda values were calculated on the basis of the range prevalence rates from the most methodologically robust studies (101 to 2250 per million) [24]. In detail, we referred to prevalence rates of 100 (lambda=940), 300 (lambda=313), 1000 (lambda=94), and 2250 (lambda=41.8) cases per million. The number of sibling pairs needed varies according to the number of genes believed responsible. In the case of adult-onset dystonia, the number of responsible genes is difficult to predict. It is likely that the condition is due to more than one susceptibility gene. It is likely that the condition is due to more than one susceptibility gene. The existence of one major gene, DYT1, accounting for a large proportion of primary early-onset dystonia and some cases of adult-onset dystonia gives support to the hypothesis of a few major genes responsible for a large proportion of disease risk.

Table 1: Number of affected sib-pairs required for linkage study based on sibling risk ratio ranging from 42 to 940 and different number of anticipated genetic loci.

	Sibling risk ratio, 940		Sibling risk ratio, 209		Sibling risk ratio, 94		Sibling risk ratio, 42	
Loci	IBD	ASP needed	IBD	ASP needed	IBD	ASP needed	IBD	ASP needed
1	0.7497	86	0.7488	87	0.7473	88	0.7440	90
2	0.7418	92	0.7327	100	0.7242	108	0.7113	122
3	0.7225	107	0.7078	126	0.6951	143	0.6779	173
4	0.7049	130	0.6842	161	0.6718	186	0.6516	240
6	0.6701	190	0.6475	254	0.6326	315	0.6170	405

Study of association with BDNF polimorphism val66met

Genomic DNA was extracted from whole blood of patiens with primary adult cranial and cervical dystonia and age-matched healthy volunteers. The Val66Met SNP of the BDNF gene was typed by PCR using primer sequences 5'-ACTCTGGAGAGCGTGAAT-3' and 5'-ATACTGTCACACACGCTG-3'. PCR products were analyzed by denaturing high performance liquid chromatography ; the patient's chromatograms for the amplified fragment were comcompared with correspondent normal control previously sequenced. The distribution of genotypes for case and control populations was tested for the Hardy–Weinberg equilibrium by a chi-squared analysis [25]. The associations between risk of CD and both genotype (Val/Val, Val/Met, Met/Met) and presence/absence of the rare Met allele were analyzed separately using chi squared test (or Fisher's exact test, where required).

RESULTS

During the study period, 260 patients with primary late-onset dystonia met the eligibility criteria and 234 (90%) participated into the study. There were 77 men and 157 women aged 62.3 + 12.6 years. Age of dystonia onset was 51.7 + 14.9 years; 134 patients presented with cranial dystonia and 100 with extracranial dystonia (age of dystonia onset 57.3 + 11.9 vs. 42.8 + 15.0 years, $p < 0.0001$). Spread of dystonia could be accurately assessed in 173 /234 patients: there were 38 patients with segmental / multifocal dystonia and 135 patients with focal dystonia whose disease duration was long enough (5 years of more in patients presenting with cranial dystonia, > 10 years in patients with extracranial dystonia at onset) to make spread unlikely. In the remaining 61 patients with focal dystonia, duration of disease was not long enough to exclude future spread.

Family study

The 234 families who took part in the study provided a potential population of 978 living first-degree relatives, of whom 801 (82%) underwent the two step-procedure (Figure 1). As stated in the methods, performing examination at home allowed us to examine not only the 115 relatives who screened positive on the questionnaire (51 were affected on examination) and the 64 who screened negative and had < 8 years of schooling (4 were

affected on examination), but also 167 relatives screening negative who had > 8 years of schooling (none was affected on examination). Overall 125/234 families had at least one family member other than the proband directly examined.

Our procedure yielded a diagnosis of dystonia in 55 relatives from 50 families. Affected relatives were assigned a diagnosis of primary dystonia because neurological examination was otherwise normal, and no causes for dystonia could be detected. Among proband-relative pairs, 36 (65%) were phenotypically discordant, and 19 (35%) were phenotypically concordant. Overall, the rate of family history of dystonia in our series was 21% (50/234).

Familial vs. sporadic patients

Comparing the demographic and clinical features of patients with positive family history (both probands and affected relatives) with those of sporadic patients yielded no significant difference in the examined parameters. (Table 2). Likewise, familial and sporadic patients reported similar frequency of eye disease and coffee drinking. Spread of dystonia was assessed in 205/289 patients, of whom 46 had positive family history. Eighty –four patients Kaplan-Meier survival curves and Cox analysis suggested that familial patients were not more likely to have spread than sporadic ones (Figure 2, Table 3). Study power was > 80%.

Genetic study

Linkage analysis: the linkage analysis using ASP method could be performed only between siblings. From the 55 relativies found affectet only 15 were siblings but this sample was not adequate for the proposed linkage analysis , therefore this genetic study could not be conducted.

Association study with BDNF polymorphism val66met: One hundred-fifty six patients with primary adult-onset dystonia (57 with focal BSP, 22 with BSP as part of a segmental dystonia, 67 with focal CD, and 9 with CD as part of a segmental dystonia) and 170 healthy controlsubjects met the eligibility criteria during the study period. The two groups did not differ for age and sex. Population heterogeneity for the Val66Met SNP was excluded amongst both control subjects ($v2 < 0.00001$, $P = 1.000$) and case patients ($v2 = 0.5162$, $P = 0.773$).Case and control subjects had similar distribution of genotype and allele frequencies . The presence of the Met allele yielded an age- and gender-adjusted OR of 1.22 (95% CI: 0.78–1.94; $P = 0.38$).

Figure:1

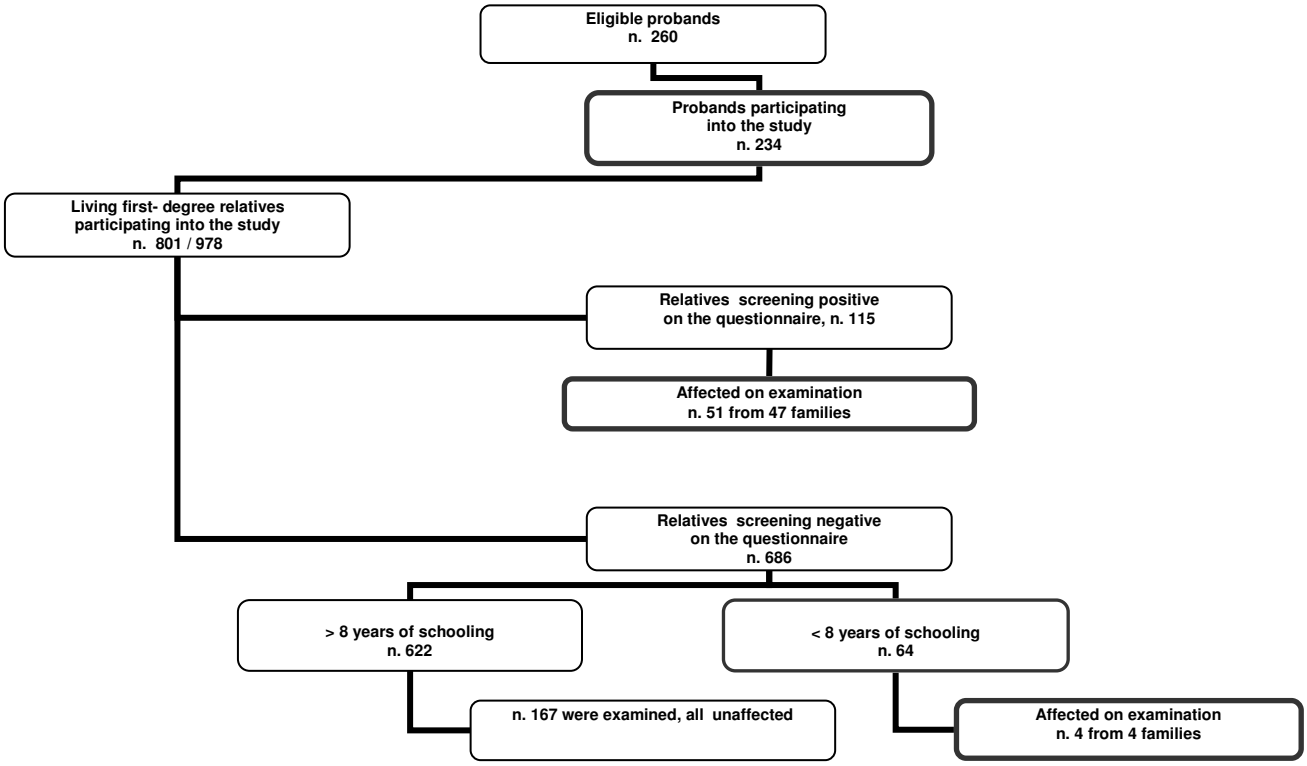

Table 2: clinical and environmental factors in sporadic vs familial patients

	Family history +	**Family history -**	**P**
N. Patients	105	184	
Age	63.6 + 12.7	61.4 + 12.5	NS
Sex (men/women)	44 / 61	56 / 128	NS
Dystonia at presentation **- Cranial** **- Extracranial**	 57 48	 101 83	 NS
Age of dystonia onset - **All** - **Cranial dystonia** - **Extra-cranial dystonia**	 52.5 + 15.9 58.6 + 13.6 42.3 + 15.5	 51.3 + 13.9 56.3 + 10.2 43.6 + 15.0	 NS NS NS
Eye diseases	40%	36%	NS
Coffee drinking	72%	69%	NS

Figure 2

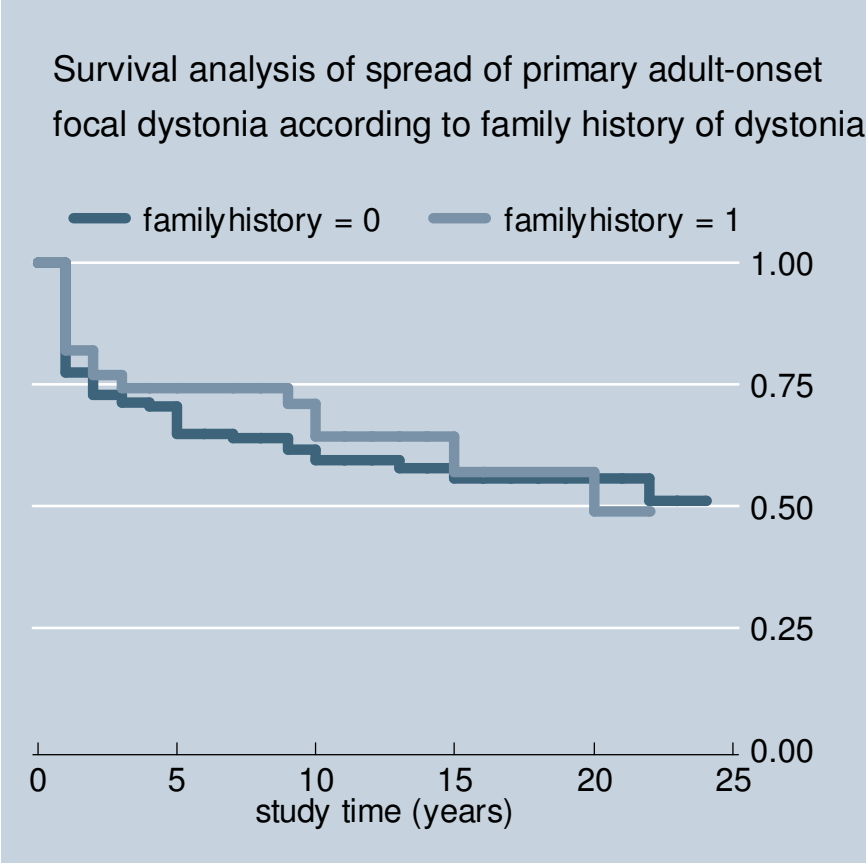

Table 3

	Hazard ratio	95% CI, P
Family history of dystonia	**1.003**	**0.6 to 1.7, 0.77**

Adjusted for referral center, sex, age of dystonia onset and type of dystonia

DISCUSSION

This study comes from a very large multicenter project and provided many data about clinical and epidemiological features of dystonia. The main aim of the project was to identify novel genes and loci associated with primary AOD by a linkage analysis using the ASP method. In the plan of the study it was foreseen to find at least 100 pairs of affected siblings as it was discussed in the method section. After recruiting patients from all 9 italian movement disorder centers we could estimate the prevalence of 21% of familial cases of AOD in Italy ; this resulted from the identification of 55 affected relatives and only part of them were siblings. This sample unfortunatly was definetly inadequate for the proposed linkage analysis that finally could not be performed. The minor aim of the study about the genetic of AOD was to asses the impact of genetic polimorphisms candidate from the litterature. The presence of Val66Met SNP of BDNF gene has been assesed in cranial and cervical dystonia from this large cohort of patients ; the study did not show any significative difference in genotype and allele distribution between case and control subjects. This shows a lack of association between the Val 66Met SNP of BDNF gene and cranial AOD and may suggest that the abnormal plasticity in dystonia could be related to other genes playing a role in pathophysiology of cortical plasticity. On the basis of new evidences of the role of the A2a adenosine

receptor (A2aAR) in neurotrasmission we recently started to investigate the occurence of the polymorphism of A2aAR at the nucleotide 1976T>C in patients with cranial AOD belonging to the study. A2aAR is very expressed and in basal ganglia mainly in GABA striatopallidum projections [26], its activation gives extrapyramidal symptoms that can be reduced by receptor antagonist for A2aAR [27]. Caffine strongly blocks A2aAR while coffee drinking is a protective factor for blepharospasm occurence [6]: this may suggest some pathophysiological role of A2aAR in dystonia. This study of association between 1976T>C A2aAR polimorphysm and cranial dystonia has only recently begun and there is no preliminary result available at the moment.

Secondary aimes of the project relates to epidemiological and clinical features of AOD. The rate of family history of dystonia in our cohort of patients was 21%, this datum would confirm and support the genetic influence in dystonia pathophysiology. We studied the impact of enviromental factors and clinical elements in sporadic and familial cases of AOD. Coffee drinking is a protective factor for cranial and cervical dystonia , and anterior eye diseases is a risk factor for blepharospasm: our study did not show any difference of the occurrence of these environmental factors between familial and sporadic cases . Reguarding the clinical features recorded in this study (site of presentation, age of dystonia onset and spread) their occurrence was also compared between sporadic and

familial cases , no significant difference between the two groups was detected. All these data support the evidence of a common pathophysological background in sporadic and familial AOD.

This was a very large multicenter study that provided striking informations for the knowledge of dystonia pathophysiology. We obtained important epidemiological data confirming the combination of enviromental and genetic elements in dystonia and showing that familial and sporadic cases of AOD share almost the same clinical picture. This is strongly suggestive of a pathophysiological common drive underlying primary AOD despite of sporadic or familial origin.

We finally collected a large amount of DNA samples from our patients and their relatives that at the moment could not be assessed for the linkage analysis but they will be soon anlysed for genome-wide association studies that may have more chances to identify novel genes related to primary AOD.

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