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Tremor and Other Hyperkinetic Movements

Reviews

Key issues in essential tremor genetics research: Where are we now and how can we move forward?

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Abstract

Background: Genetics research is an avenue towards understanding essential tremor (ET). Advances have been made in genetic linkage and association: there are three reported ET susceptibility loci, and mixed but growing data on risk associations. However, causal mutations have not been forthcoming. This disappointing lack of progress has opened productive discussions on challenges in ET and specifically ET genetics research, including fundamental assumptions in the field.

Methods: This article reviews the ET genetics literature, results to date, the open questions in ET genetics and the current challenges in addressing them.

Results: Several inherent ET features complicate genetic linkage and association studies: high potential phenocopy rates, inaccurate tremor self-reporting, and ET misdiagnoses are examples. Increasing use of direct examination data for subjects, family members, and controls is one current response. Smaller moves towards expanding ET phenotype research concepts into non-tremor features, clinically disputed ET subsets, and testing phenotype features instead of clinical diagnosis against genetic data are gradually occurring. The field has already moved to considering complex trait mechanisms requiring detection of combinations of rare genetic variants. Hypotheses may move further to consider novel mechanisms of inheritance, such as epigenetics.

Discussion: It is an exciting time in ET genetics as investigators start moving past assumptions underlying both phenotype and genetics experimental contributions, overcoming challenges to collaboration, and engaging the ET community. Multicenter collaborative efforts comprising rich longitudinal prospective phenotype data and neuropathologic analysis combined with the latest in genetics experimental design and technology will be the next wave in the field.

Keywords: Essential tremor, gene, linkage, association, dystonia, parkinsonism

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Introduction

Essential tremor (ET) is a progressive and often disabling disorder:[1–4] severe kinetic tremor can destroy abilities such as eating, drinking, and handwriting. Even mild tremor may affect work skills and can be socially devastating. ET is highly prevalent, estimated at 5% for age 65 years and over.[5–8] Treatment is at the level of symptom suppression, not disease modification, and is variably effective at best.[9] Despite all these motivations, research on ET is relatively undeveloped. As the basic underlying mechanisms of ET are unknown, with controversies remaining even in which area(s) of the brain are involved, the ability to validate model systems is limited, and intelligently developing targeted therapeutics is extremely difficult.

One approach many talented groups have turned to in the hopes of breaking open the field is genetics. Recognition of frequently positive tremor family history in ET dates back to its earliest definitions, with general credit in the Western literature going to Most in 1836.[10,11] Detailed ET pedigrees appear in the literature of the 1880s.[12] Despite progress in ET genetics, causal mutations have not been forthcoming. This disappointing lack of progress has opened productive discussions on challenges in ET and specifically ET genetics research, including fundamental assumptions in the field.[13–15] This article reviews the basic open questions in ET genetics and key challenges in addressing them.

Is ET a genetic disorder?

Twin studies support a large role for genes over environment in ET.[16,17] In one study, pairwise concordance in monozygotic twins (0.60) was about double that in dizygotic twins (0.27).[17] Another

study[16] noted a significant shift in concordance rates depending on the use of Tremor Investigation Group (TRIG)-defined possible, probable, or definite ET cases (see Deuschl et al[18]): including only probable and definite cases, the concordance rates were 0.93 monozygotic twins and 0.29 dizygotic, putting the heritability for potential development of ET at up to 99% using conservative prevalence estimates in an aged population.

ET subjects generally self-report a positive tremor family history over 60% of the time, but study results range widely, from 17% to 100%.[10,12,19–24] Methodology is one factor. Only a fraction of ET subjects seek medical attention; therefore, studies using clinic-based versus community-based approaches may obtain different estimates of positive ET family history due to selection biases.[22] An ET subject with affected family members may be more likely to seek medical attention having observed potential treatment plans, or less likely if multiple family members have been told treatment is ineffective or unimportant. In specific communities where cases can be directly ascertained and there are detailed inheritance records, ET appears strongly familial. For example, a study of a Swedish parish traced all but 2 of 210 ET cases back to four ancestral couples, with an autosomal dominant inheritance pattern.[25] In addition, using direct contact with family members rather than proband recall of family history can greatly change results. Busenbark et al[26] used a series of escalating data steps (proband recall at a clinic visit, mail and phone contact with probands, mail contact with first-degree relatives) to increase an initial 68% positive family history rate to 96% in 253 US ET cases. The true rate of familial ET remains unclear.[22,27–29]

Progress and issues to date: genetic linkage and association studies

Approaching ET as a genetic disorder, work to date has created consensus that ET likely represents a family of disorders. As discussed in the next section, the concept of ET as genetically heterogeneous has shifted to a complex genetics structure, assuming many genetic changes come together to trigger disease pathology, although rare Mendelian monogenic forms of ET may also exist. This section reviews ET genetic linkage and association studies to date, which have largely operated under a more basic framework, wherein a few Mendelian monogenetic changes create a family of similar phenotypes. We have seen some progress towards identifying causal mutations or genetic risk factors for ET. Inherent features of ET as currently defined directly impact progress in genetics studies.

Genetic linkage

Genetic linkage experiments are based on the idea that stretches of chromosomes tend to be inherited together. Alleles (groups of genetic markers) or loci (areas of DNA containing many genes) that are "linked" tend to stay together across meiosis because they are on the same chromosome, and are close enough together that chromosomal recombination during meiosis is unlikely. The farther apart alleles are, the more likely that DNA crossovers during meiosis will separate the alleles over time. Linkage experiments for ET hypothesize that a locus observed in ET family members and not unaffected family members is linked to an as-yet unidentified causal genetic change.

The logarithm base 10 of odds (LOD) score[30] is a statistical test for linkage analysis, which compares the likelihood of results if two loci are linked to the likelihood of observing the data if two loci are not linked. A LOD score greater than 3.0, i.e., 1,000 to 1 odds of the result observed if the loci are linked, is by convention evidence for linkage. LOD scores may be added up across multiple families to achieve a final score. Another measure of genetic linkage is recombination frequency (θ). This is the frequency at which DNA crossover takes place between two genetic areas during meiosis. It can therefore be thought of as a measure of distance: the farther away two alleles are, the more likely crossover events will occur and separate them over time. A centimorgan (cM) is a genetic distance unit of 1% recombination frequency; this is a useful estimate of real distance. As distances increase, double crossovers become more possible, negating the ability to detect recombination and causing a systematic underestimation of genetic distance; one critique of current ET loci is that some reports put them in this potential size range.

There are three distinct reported susceptibility loci linked to ET under autosomal dominant models (Online Mendelian Inheritance in Man, www.omim.org): ETM1 on chromosome 3q13 (OMIM 190300), identified with a genome-wide scan of 16 Icelandic families using TRIG-defined definite (classic) ET as affected,[31] and confirmed in four unrelated Tajik families;[32] ETM2 on 2p24 (OMIM 602134), linked in an American (Czech descent) family;[33–35] and a chromosome 6p23 region linked in two North American families with ET plus dystonia but not families with classic ET alone (OMIM 611456).[36]

Only ETM1 has been independently confirmed.[32] The original Icelandic study, assuming an autosomal dominant model, reported a parametric analysis LOD score of 3.71, or non-parametric Z score of 4.70, $p<6.4\times10^{-6}$.[31] Of note, the single pedigree LOD scores were 1.29 and below. The Tajik linkage study yielded a maximum pairwise LOD score of 2.46 and maximum combined multipoint LOD score of 3.35; the authors argued for narrowing the locus from the original (large) 10 cM range to 2 cM.[32] An initial ETM2 follow-up DNA sequencing study of Korean individuals reported sequence variants within ETM2 found in 23 classic ET but not seven "non-classic" ET or 30 controls.[37] A subsequent association study of three polymorphic markers within ETM2 in a Czech cohort was negative,[38] as was a case–control study of microsatellite markers across ETM2 in a Latvian cohort.[39] The only published ETM3 follow-up confirmation attempts are locus exclusions in some ET families.[36,40,41] This may reflect the low parametric LOD scores from the two ETM3 pedigrees, and the observation of multiple instances of non-penetrance in the haplotype analysis.[36] It may also reflect an ET–dystonia connection rather than genetic heterogeneity in "classic" ET (see below). More ET loci exist but await identification, as ETM1 and ETM2 have been excluded in other genetically informative ET families.[32,36,41–45] Thus, ET is a genetically heterogeneous disorder: multiple genetic loci contribute to similar clinical phenotypes.

Genetic risks: association studies

Genetic associations provide correlative data, key starting points for determining causal links between genetic changes and disease, particularly in entities like ET with limited mechanistic information. Associations of genetic variants with ET may provide connections to or further separation from other disorders. Results on associations between genetic variants and ET risk have been mixed (Table 1), but they add to genetic linkage data suggesting mechanistic heterogeneity within clinically defined ET.

A genome-wide association study (GWAS) in ET observed association with single nucleotide polymorphisms (SNPs) in the leucine-rich repeat- and Ig domain-containing Nogo receptor-interacting protein 1 gene (*LINGO1*) on chromosome 15q (OMIM 609791), outside reported ET-associated loci. The study used an Icelandic cohort with 452 ET and 14,394 controls, then confirmed one SNP (rs9652490) in Icelandic, US, and European ET cohorts.[46] Reported odds ratio for rs9652490 was 1.54 with a population attributable risk of approximately 20%. The *LINGO1* result has been replicated in multiple independent samples,[14,47] including European,[48] North American,[49–51] Latvian,[52] and Asian[53] cohorts. At least one study used only definite ET cases.[48] In one study, restricting cases to probable or definite, not possible ET, or restricting analysis to early ET onset (age<40) strengthened the association observation.[51] Multiple *LINGO1* SNPs have been proposed over the range of studies, with the most consistent data behind rs9652490. Collectively, association studies suggest that the *LINGO1* SNP rs9652490 confers modest increased risk for ET, with odds ratios in the range of 1.2–1.7 across multiple studies and populations (Table 1).

Some *LINGO1* studies did not replicate an association with ET, or observed mixed results. Neither SNP from the original GWAS was associated with risk of ET in Chinese Han,[54] Spanish,[55] or French Canadian[56] cohorts. A separate Chinese study found no significant association of rs9652490 with ET;[57] however, a meta-analysis combining the initial data with published[53] and unpublished data from another cohort suggested increased ET risk with the rs9652490 G allele in a logistic regression analysis, under a possible recessive model for *LINGO1* ET inheritance.[57]

The overall results in *LINGO1* could be due to population-specific differences,[14] statistical issues such as multiple testing and sample sizes, or issues in ET genetics as discussed below. Alleles (DNA sequences) can be combined into haplotypes. Linkage disequilibrium, meaning a non-random association between alleles at multiple loci, is the observation of allelic combinations more (or less) often than expected in a population, compared with haplotypes formed randomly influenced only by allele population frequencies. Population-specific differences thus include allelic heterogeneity; haplotype structure variability; and differences in linkage disequilibrium patterns. *LINGO1* has roles in oligodendrocyte development and axonal regeneration. However, it is unknown if *LINGO1* has any functional role in ET: the GWAS SNP is intronic, and may be in linkage disequilibrium with an actual functional genetic variant.[13,14]

Given the *LINGO1* findings, some groups have explored sequence variation and risk associations of related genes. A multicenter North American effort utilizing 1,247 ET and 642 control samples combined gene sequencing, association studies, and SNP haplotype tagging across *LINGO1* and the paralog *LINGO2* to identify one *LINGO2* SNP associated with risk for ET under a recessive model (rs1412229).[49] In addition, two haplotype-tagged SNPs in *LINGO2* (rs10812774, rs7033345) influenced ET age of onset.[49] A separate study in two Asian cohorts comprising 327 total ET and 499 controls identified different *LINGO2* SNPs associated with ET (rs10812774) or ET and Parkinson's disease (PD) (rs7033345) risk, under recessive models.[58] Reported *LINGO2* risk associations are significant but relatively weak. Potential associations with ET and genetic variants in the homolog *LINGO4* were not detected in 150 Chinese Han ET and 300 control samples.[59]

While *LINGO1* remains the strongest genetic risk finding for ET to date (even with the overall mixed results), it is outside the reported ET loci. The first reported specific ET-associated genetic variation, 828C→G in the HS1 binding protein 3 gene (*HS1-BP3*), is within ETM2.[60,61] While initial work pointed to a strong association between this polymorphism and at least some ET,[60,61] subsequent studies did not confirm this association, arguing for at best a restricted role, perhaps as a region in close linkage disequilibrium with a causative mutation in some families.[62,63] The *HS1-BP3* 828C→G variant was not associated with either ET or PD risk in our cohorts.[64] Within ETM1, the dopamine D3 receptor (DRD3) gene (*DRD3*) 312A→G variant is implicated in small, French ET families and in a case–control analysis of US subjects: *DRD3* genotype was associated with age of onset and disease severity in these samples;[65] an independent study of Spanish subjects also observed an association with both ET risk and ET age of onset.[66] In contrast to these reports, replication studies in Asian, Latvian, German, French, Danish, Italian, and at least two independent US ET cohorts did not observe an association between *DRD3* variants and ET risk or ET age of onset,[39,64,67–70] or linkage with *DRD3* in ET families.[67,69] *DRD3* is unlikely to play a role in ET genetic pathophysiology.

There are numerous negative association studies based on potential ET-like phenotypes within other disorders such as PD (including leucine rich repeat kinase 2 *LRRK2*, and glucocerebrosidase *GBA*), fragile X, and spinocerebellar ataxias, as well as a few unconfirmed positive reports (microtubule-associated protein tau *MAPT* for example).[45,71–74] The discussion below focuses on hypotheses specific to ET, and studies with independent follow-up.

One study observed an association between the length of a mixed dinucleotide repeat sequence (REP1) in the gene encoding α-synuclein (*SNCA*) and ET as well as PD.[75] *SNCA* mutations and genomic multiplications play a clear role in rare forms of inherited PD.[76–82] REP1 allele length variants are associated with idiopathic PD in multiple studies.[75,83–89] However, a subsequent independent study of *SNCA* and ET (Italian cohort) did not find an association between ET risk and *SNCA* haplotypes.[90] This study was unable to directly assess the REP1 finding for various reasons, including issues with interpreting

Table 1. **Association Study Cohort Sizes in Essential Tremor (ET) Genetics Research**

Study	ET n	Control n	ET Cohort Source	Overall Result
ETM1				
HS1-BP3 828C→G variant Shatunov et al, 2005[63]	49	92	United States	Negative
HS1-BP3 828C→G variant Higgins et al, 2006[61]	73	304	United States	Positive Note initial 2005 report from same group with G variant in ET cases in 2 of 21 US ET families, not ET or unaffecteds in the other families, and not 150 US controls or 73 ET Singaporean cases
HS1-BP3 828C→G variant Deng et al, 2005[62]	222	132	United States	Negative
ETM2				
ETM2 sequence variants Kim et al, 2005[37]	30	30	Korean	Positive
ETM2 polymorphisms Zahorakova et al, 2010[38]	61	68	Czech	Negative
DRD3 312A→G variant Inashkina et al, 2008[39]	104	116	Latvian	Negative
DRD3 312A→G variant Vitale et al, 2008[70]	116	158	Italian	Negative
DRD3 312A→G variant Tan et al, 2007[68]	163	192	Singaporean	Negative
DRD3 312A→G variant Garcia-Martin et al, 2009[66]	201	282	Spanish	Positive
DRD3 312A→G variant Jeanneteau et al, 2006[65]	276 30	184 50	United States French	Positive
DRD3 312A→G variant Lorenz et al, 2009[69]	299	528	German, Danish, French	Negative
DRD3 312A→G variant Blair et al, 2008[67]	433	272	United States	Negative

Table 1. Continued

Study	ET n	Control n	ET Cohort Source	Overall Result
LINGO genes				
LINGO1 rs9652490 and rs11856808 Zuo et al, 2010[54]	109	430	Chinese Han	Negative
LINGO1 rs9652490 Wu et al, 2011[57]	117	160	Chinese	Negative Note combining with Tan et al[53] plus unpublished data into 307 ET and 804 control, meta-analysis positive
LINGO1 rs9652490 Radovica et al, 2012[52]	141	130	Latvian	Positive 9 other SNPs including rs11856808 negative
LINGO4 variants Liang et al, 2012[59]	150	300	Chinese Han	Negative
LINGO1 rs9652490 Tan et al, 2009[53]	190	733	Singaporean	Positive
LINGO1 rs9652490 and rs11856808 Lorenzo-Betancor et al, 2011[55]	226	1117	Spanish	Negative
LINGO1 SNPs Clark et al, 2010[51]	257	265	United States	Positive for rs9652490 and others Negative for rs11856808 and others out of 15 SNPs total Note meta-analysis combining 3 or 4 published cohorts[46,50,51,53] also positive
LINGO1 rs9652490 and rs11856808 Bourassa et al, 2011[56]	259	479	French Canadian	Negative
LINGO2 variants Wu et al, 2011[58]	327	499	Chinese, Singaporean	Positive for rs7033345 and rs10812774 of 8 variants
LINGO1 SNPs Thier et al, 2010[48]	332	574	German, French	Positive for rs9652490 and 1 other Negative for rs11856808 and others out of 10 SNPs total
LINGO1 rs9652490 Vilarino-Guell et al, 2010[50]	356	428	North American	Positive

Table 1. Continued

Study	ET n	Control n	ET Cohort Source	Overall Result
GWAS Stefansson et al, 2009[46]	452 281	14,394 1188	Icelandic Austrian, German, United States	Positive for *LINGO1* rs9652490 and rs11856808 in initial cohort; rs9652490 only in confirmatory cohorts
LINGO1 and *LINGO2* variants Vilarino-Guell et al, 2010[49]	1247	642	North American	Positive for *LINGO1* rs9652490 and 4 others of 16 total, and *LINGO2* rs1412229 alone of 21.
SNCA				
REP1 length Tan et al, 2000[75]	46	100	United States	Positive
SNCA variants Pigullo et al, 2003[90]	106	90	Italian	Negative
SNCA variants Ross et al, 2011[91]	647	1285	North American	Negative
Other variants				
GABAAR subunit genes variants Garcia-Martin et al, 2011[94,95]	200	250	Spanish	Negative
GABAAR subunits and GABA transporter genes variants Thier et al, 2011[93]	503	818	German, Dutch	Negative
HNMT rs11558538 Ledesma et al, 2008[96]	204	295	Spanish	Positive
HNMT rs11558538 Keeling et al, 2010[97]	338	409	North American	Negative

Studies are grouped under genetic categories, and listed by first author and year of publication. Within hypothesis groups, studies are ordered by ET n. If the study combined cohorts in one analysis then the full ET n is used. If the same study used different cohorts in different analyses, the larger ET n is listed first. ET cohort source is geographical; some papers have further details on ethnicity. North American = United States and Canada. Overall result considers the main thrust of the study, with a few further notations; see text for comments on age of onset influences, and cited references for full study results details including specific genetic variants tested, haplotype analyses, methods.

Abbreviations: *DRD3*, dopamine D3 receptor gene; ET, essential tremor; ETM, Hereditary Essential Tremor, Online Mendelian Inheritance of Man (OMIM) locus designation; GABA, gamma-aminobutyric acid; GABAAR, gamma-aminobutyric acid A receptor; *HNMT*, histamine *N*-methyltransferase gene; *HS1-BP3*, HS1 binding protein 3 gene; LINGO, leucine-rich repeat- and Ig domain-containing Nogo receptor-interacting protein; numbered italicized *LINGO*s are genes encoding LINGO proteins; REP1, mixed dinucleotide repeat sequence in the *SNCA* promoter region; *SNCA*, gene encoding α-synuclein; SNPs, single nucleotide polymorphisms; US, United States.

REP1 lengths: the four dinucleotide repeats constituting REP1 all varied; thus, alleles with identical lengths may represent varied dinucleotide sequences instead of homozygous alleles.[90] An extensive study of SNPs across the *SNCA* locus in 647 ET and 1,285 control samples did not show any associations with ET risk, although the previously reported *SNCA* risk association with PD was replicated.[91]

A mouse model of ET in gamma-aminobutyric acid A receptor (GABAAR) alpha-1-subunit −/− mice has been proposed based on face validity of the mouse phenotype.[92] Subsequent human subject studies have tested GABA and GABAAR genetic variants against ET risk. Polymorphisms in 15 GABAAR and four GABA transporter genes were investigated in German and Dutch cohorts. No evidence for association with ET risk was observed.[93] Variants in GABAAR subtype genes were also not associated with ET risk studies in Spanish samples.[94,95]

The histamine *N*-methyltransferase gene (*HNMT*) rs11558538 threonine allele, a functional variant, was nominated as an ET risk factor.[96] This result was not observed in a follow-up study by an independent North American group.[97]

Challenges for genetics studies to date

Several inherent ET features complicate genetic linkage and association studies (Table 2). This section reviews issues of phenocopies and type 1 error, age dependence of phenotype expression and type 2 error, tremor self-reporting, ET misdiagnoses, ranges of phenotype that include mild tremor, and tensions between study feasibility, sample size, and power.

High prevalence that increases with age and multiple distinct susceptibility loci, together with a lack of information on possible environmental determinants, all increase the possibility of phenocopies within a family.[22,36,98] Phenocopies are people with a similar or identical phenotype due to different underlying genotypes or environmental factors. Genetic linkage is based on the idea that a given trait (phenotype) segregates with a distinct area and variant of DNA (genotype). Even a single phenocopy has a large impact on the LOD score, since in at least that phenocopy case (and possibly also their descendants) the ET phenotype will not segregate or "link" to the same locus as in the rest of the family. Given a 5% prevalence rate, older family members will have a 1 in 20 possibility of ET by chance alone. Spouses marrying in to the family have that same possibility of having ET. There is already evidence for multiple loci in ET, making it difficult to assume that spouses or even related affected family members are not phenocopies. In association studies, phenocopies represent false positives in the cases, potentially increasing type 1 error.

The age dependence of phenotype expression in ET has further implications. Subjects of any age without tremor may simply be presymptomatic. Controls too young to express their ET phenotype become false negatives in association studies. Given that prevalence is thought to increase with age, restricting controls to older ages at least decreases this source of false negatives and type 2 error, although it does not eliminate it. Incorrect unaffected status assignments in linkage studies decrease the LOD score, much like unrecognized phenocopies.

Assuming no phenocopies within a family or case group, several issues still directly impact results (Table 2). Early studies often relied on self-reporting for tremor diagnosis, particularly for family members thought to be unaffected. Self-reported family history may have poor validity,[99] and tremor self-reporting is often inaccurate;[7,10,100–102] thus, direct examination of all family members and controls is necessary for accurate phenotype assignments. Self-reporting can introduce error via misdiagnoses of significant tremor, and non-reporting of mild tremor, as discussed below. Direct examinations of all subjects are critical for data quality, but can be affected by ascertainment bias when not blinded. On the other hand, individual research efforts may not have the personnel to conduct assessments blinded to subject status as a proband, family member, or unrelated control. Acquiring any examination data, especially on controls, may be resisted on the basis of cost. This is partially connected to a low awareness of the importance of acquiring any tremor data in controls: the current National Institute of Neurological Disorders and Stroke (NINDS) Coriell repository control data set lists "essential tremor" under Other Medical History for two of over 3,000 controls, well below population-based prevalence estimates of 5%, and far below 23% mild undiagnosed ET in a study of ostensibly healthy older controls.[7] However, there is no specific "tremor" personal or family medical history question for the NINDS repository, despite PD and dystonia questions, and a parkinsonism case collection.[103] PD studies will also suffer from lack of any basic systematic tremor information on controls, as below.

Older family members are more likely to be taking medications, potentially causing tremor, or have medical comorbidities like thyroid disorders that cause unrelated tremors. More challenging are the frequency and type of ET misdiagnoses. ET is a misdiagnosis in up to 30–50% of cases.[104,105] The most common alternative diagnoses in these series were PD and dystonia.[104,105] Conversely, ET is frequently misdiagnosed as PD.[23,106] In the classic Hoehn and Yahr[107] PD study, 39 of 856 subjects were excluded after determination of ET as their correct diagnosis. Parkinsonism was incorrectly assigned to over half of the cases in one nursing home study, based on a movement disorders specialist review.[108] Many patients carry a misdiagnosis for years.[109] Setting aside current controversies in potential connections between ET, PD, and dystonia, ascertaining a solid ET clinical diagnosis contains enough uncertainty to potentially generate significant false-positive and false-negative results, particularly when subject self-reporting is used. Direct examinations using in-person movement disorders expertise, along with video-recording as primary data instead of examiner recall alone, can help reduce these error rates and greatly improve genetics study data quality. As above, this high bar for phenotyping data comes with costs in personnel and subject time.

In assigning status for genetic experiments, clinically relevant tremor is only part of the spectrum of research relevant tremor. On the lower end of tremor severity, it is impossible to distinguish enhanced physiologic tremor (considered in the range of normal) from very mild ET. ET subjects may transition from minimal hand tremor to noticeable ET-like tremor without electrophysiological evidence of 8–12 Hz physiologic tremor, implying that the initial tremor represents early ET rather than a separate entity.[110] Research definitions may conflict with community needs and understanding: family members with mild ET may self-report no tremor, or at least resist an affected label as they do not consider themselves to have a clinical diagnosis. At the same time, tremor in ET has long been recognized as progressive.[10,25] This includes the phenomenon of longstanding mild

Table 2. **Consequences of Basic Essential Tremor (ET) Features Create Challenges for ET Genetics Research.** The current ET clinical definition has a direct impact on ET research. Ways to move forward acknowledge and address these challenges.

Current ET Feature	Consequences	Possible Responses	Challenges	Moving Forward
Phenocopies: High prevalence Multiple genetic loci Unknown genotypes	Creates false positives Lowers LOD score Increased type 1 error One genetic variant may have large phenotypic range (genocopies).	Test different phenotype features or subsets against genotype data (see also Table 3). Use inclusive varied phenotype as outcome rather than narrow ET definition.	How to make phenotype groups more genetically homogeneous is currently unknown. Expanding or narrowing possible phenotypes may or may not increase genetic homogeneity within groups.	Research definitions, specify ET subsets, accept potential even "non-ET" phenotype variables as part of hypotheses generation and testing, use phenotype variables across clinical diagnoses. Experimental design goals are to broaden testable hypotheses, thus move past the potential stalemate of as yet unknown phenotype-genotype connections.
Increasing prevalence with age	Creates false negatives Lowers LOD score Increased type 2 error	Consider all familial unaffected subjects as unknown. Restrict controls to older ages.	Strict affected status assignments can greatly impact power. Lowers impact of this error type but does not eliminate it.	Prospective studies of age of onset may change perspective on this ET feature. Large collaborative efforts particularly on control data and family member data help improve power and get past this ET feature.
ET is a *clinical diagnosis*:		Prospective longitudinal primary exam data	Detailed longitudinal phenotyping requires high investment in time and funds.	Collaborative efforts between: multiple centers; movement disorders experts and genetics experts; researchers and ET research participants.
Tremor self-reporting	False negatives	Direct exams of all subjects: case, control, family member	Both subject and researcher resources impact feasibility of extended direct exams.	Collaborative efforts to define minimal and additional phenotype data, minimal and additional biosample amounts and time points.
Lack of any tremor data particularly for controls	False negatives			
ET misdiagnoses: Other disorders; mild tremor versus normal	False positives and false negatives	Larger sample sizes to improve ability to detect associations Utilizing possible / probable / definite categories, restricting analysis to definite cases	Large sample sizes are expensive and time consuming to collect. There is always uncertainty in a clinical diagnosis, especially with mild tremor, and comparing to other clinically defined movement disorders. Some level of mechanistic heterogeneity likely in all case and control groups.	Explicit review of feasibility versus data quality tradeoffs to understand choices for each experiment: Non-blinded exams may cause ascertainment bias; time burden for subjects may limit phenotype data; scoring videos rather than in person exams may lower some phenotype data quality but improve blinding and allow multiple examiners; definite-only ET case definition may strengthen result but impact power. Use experimental design process to broaden range of testable hypotheses as above.

Abbreviations: ET, essential tremor; LOD, logarithm base 10 of odds.

hand tremor morphing after years to decades into symptomatically significant typical ET that prompts diagnosis and treatment.[10,25,111] In our own research cohort, 12 of 38 members in one family self-reported no tremor but had tremor on examination, including cases of definite ET and cases who would acknowledge "nervousness" rather than tremor.[112]

One approach is to utilize a possible/probable/definite scheme for ET research diagnosis,[18,113] as in the twin study above.[16] At least three of the association studies above restricted ET cases to TRIG criteria definite[48,90] or probable and definite ET.[91] This has obvious implications for achieving higher sample size case groups (Table 2).

The tension between reducing false-positive/negative rates, ET research diagnoses, and feasibility considerations can be seen in Table 1, a listing of association studies by general genetic category and ET cohort size. While control group sizes also matter, in most studies there is even less information on controls than cases. With the exception of *LINGO* genes, in general small sample size studies report a positive result that over time moves to larger sample sizes and negative outcomes. Note also larger cohorts are weighted to Western European and North American sources, which are heavily self-reported Caucasian or White, with limited ethnic variability, although there are some data on Ashkenazi Jewish subjects and rare African-American subjects.[51,91]

The Shatunov et al[36] ETM3 linkage study restricted affected status to definite ET in directly examined subjects, and considered all other family members unknown. This stringent approach deals with age dependence of tremor expression, and the uncertainties in both mild tremor and ET misdiagnosis (Table 2). It also imposes a high burden on achieving enough power to detect a potential locus.

Fundamental assumptions and ET genetics research

Many genetics studies report no information on how ET is determined, little information on subject cohort characteristics beyond number of ET versus controls, and little to no information on how terms like "familial" and "sporadic" ET are defined. Linkage experiments vacillate between lack of solid data for accurate subject status assignments and loss of power; association studies often use very small sample sizes and less expensive but less informative techniques like studying a single SNP. These issues, discussed above, impact the ability to detect ET causal mutations and genetic risk associations in studies to date (Table 2). We can do better on the basics: many groups already are. Is that enough? Conceptualizing ET as a family of primarily genetic disorders and improving the quality of basic work has moved the field forward, but only so far. Past ET genetics studies largely work under two sets of assumptions: definition choices framing ET as a phenotypically homogeneous disorder; and genetics experiment structures based on straightforward Mendelian autosomal dominant inheritance of common genetic variants. Current key challenges involve shifting fundamental assumptions to create further progress in the field (Table 3).

From the movement disorders side: defining the ET phenotype

Even the best ET work contends with assumptions underlying all clinically defined complex disorders: the clinical definition is known, and the clinically based definition relates to underlying pathology. An excellent treatment by MacMahon and Pugh[114] notes "The disease entities … have been selected, from the innumerable possibilities … on the basis of usefulness for prevention or treatment or on the basis of medical tradition." To begin to organize clinical care, definition decisions must be made based on disease manifestation, which is sometimes a matter of tradition rather than data. One only assumes that "arrangements of ill persons by their manifestations may identify groups that have at least some degree of homogeneity with respect to causal factors … a useful basis for investigation of cause."[114]

The spectrum of ET phenotype and pathology are still open questions, creating opportunities to re-examine current clinical ET definitions, particularly that of a monosymptomatic homogeneous clinical picture.[18,115,116] Even a clinically useful definition is not necessarily useful for research on disease mechanism. Phenotype definition issues relevant to ET genetics research include whether and how to use tremor age of onset, motor symptoms beyond action tremor, non-motor symptoms, and phenotypes closely overlapping with other clinically defined movement disorders.

Starting with monosymptomatic kinetic tremor as ET, a proposed ET research variable is early age of onset. As an example, LINGO gene variants may influence ET age of onset, or be more strongly associated with early age of onset cases.[14,49,51] However, retrospective age of onset represents fairly weak data in ET. Patients often report the age when tremor became noticeable or bothersome as their age of onset, discounting the "nervousness" or mild tremor noted from a young age.[10] Prospective age of onset studies will require extended longitudinal follow-up of large varied cohorts. In the meantime, clarifying ways to obtain age of onset as research data, or working within the small subset of cases with moderate to severe tremor at an early age, could strengthen the use of this proposed characteristic.

The debate becomes more interesting when research moves away from monosymptomatic kinetic tremor. Are there other motor, and non-motor, ET features? Are there mechanistic connections between ET and other disorders? These are areas of intense opinion. They are considered here in the context of ET genetics research design, advocating moving past binary clinical label phenotype data (Table 3).

The idea of formally dividing ET into classic and "complicated" cases, usually ET–parkinsonism or ET–dystonia, dates back to at least the 1800s.[10,115,116] Early attempts to clearly separate ET and PD also acknowledge "exceptional cases" that could represent an ET–parkinsonism overlap.[10] Researchers may therefore have to make definition choices discordant from clinical ones, or be more agnostic about which features are "allowed" in research phenotypes. Exploring ET in a research context may require using ET subsets within or beyond clinical diagnoses. Whether or not clinical ET exists in subsets, use of ET subsets and broader phenotyping parameters may be useful for research.

Table 3. **Moving Past Underlying Assumptions in Essential Tremor (ET) Genetics Research.** Many research groups are responding to early assumptions about ET with new approaches and theories. This process uncovers further challenges to advancing ET genetics research.

Assumption	Possible Responses	Challenges	Moving Forward
ET is a simple, known phenotype	Collect range of motor and non-motor phenotype data	ET is tremor in isolation by clinical definition	Use of research phenotype features rather than clinical diagnosis criteria
Basic binary ET / no ET phenotype groups are adequate for genetic analyses	"Sporadic" versus "familial"	May not be currently useful, often ill defined.	If "familial" subset used, clear definition with how family history data obtained.
	Early age of onset	Retrospective data, large differences in actual tremor age of onset versus bothersome increased tremor symptoms reported as age of onset.	Longitudinal prospective data would strengthen this considerably.
	Classic ET versus complicated ET	ET is tremor in isolation by clinical definition	Use of research phenotype features rather than clinical diagnosis criteria.
	ET-PD	ET and PD may or may not be related disorders; ET and PD are common mutual misdiagnoses.	Record ET, PD, and dystonia exam features; then able to exclude ET-PD or dystonia cases from genetics studies, or
	ET-parkinsonism	ET is tremor in isolation by clinical definition; ET and PD may or may not be related disorders	focus on an ET-PD, ET-parkinsonism, or ET-dystonia subset depending on hypothesis.
	ET-dystonia	Isolated head tremor is ET by clinical definition, but could be cervical dystonia; ET and limb dystonia misdiagnoses versus ET plus dystonia in non-tremor area.	Longitudinal prospective studies with neuropathology will best address research questions.
ET is a family of autosomal dominant disorders caused by a small number of common genetic variants	Many rare genetic variants, alone and in combination, are behind much of ET.	Detecting different types of genetic risk factors and causal mutations	Collaborative phenotype-genotype studies with multiple research groups to achieve large sample numbers and rich prospective longitudinal phenotype data.
	Epigenetic or other novel hypotheses for disease transmission	Addressing non-mendelian inheritance at a complex biological data level	High-throughput next generation genetic sequencing (exome, genome), epigenetic (methylome) and data analysis techniques.
ET research subjects do not want to or need to understand genetic research at its current level, as no causal mutations are known.	ET community research concerns may strengthen phenotyping and genetics experiment approaches.	ET community concerns may not be relevant to phenotype-genotype experiment design.	Understand ET community research goals, ET subject observations about ET phenotype and inheritance.
	Return genetic research results information to research subjects and ET community.	How to return complex genetic data information to research subjects is not straightforward.	Improve education of ET community on goals and results of genetics research, to increase motivated informed participation in genetics research studies.

Abbreviations: ET, essential tremor; PD, Parkinson's disease.

The ongoing attempts to untangle ET and PD[117–119] can be viewed from a genetics research perspective. Pragmatically, cases with both ET and PD (ET–PD) are well reported. Whether ET–PD represents individuals fortunate enough to have two common entities by chance, or one underlying mechanism evolving from an ET to an ET–PD phenotype, specifying how parkinsonism and PD is determined in ET cases and eliminating ET–PD cases from ET groups is important (see Ross et al[91] for one example) but far from standard in ET genetics work.[118] Focusing specifically on ET–PD compared with ET or PD alone, or ET–PD families rather than cases,[120] may help settle points of debate. Research groups are already looking for genetic connections between ET and PD, at both association and family study levels, with mixed results.[15,49,91,118–122] Specific families with apparent coinheritance of ET and other defined disorders, such as PD,[120] PD and restless leg syndrome,[123] or idiopathic normal pressure hydrocephalus,[124] may yield rare variant information.

Features beyond kinetic tremor may be part of the ET phenotype itself, not an indication of ET plus a second disorder. Some level of parkinsonism, not PD, may be clinically acceptable in ET. Minimal parkinsonian signs without clinical PD are frequently reported, including mild changes in tone with cogwheel rigidity, and mild arm swing decrease.[23,25,112,115,116] Severe kinetic tremor may break up fine motor tasks, creating clumsiness symptoms and impaired fine motor testing. Rest tremor is documented in ET, notably in individuals with longstanding severe classic ET in whom the rest tremor component is less severe than kinetic/posture tremor.[112,125–127] Rest tremor in ET may be observed without any other parkinsonian signs.[112,125–127] Specifying parkinsonism features as part of minimal ET phenotyping allows investigators to test a range of hypotheses: that all ET cases share an underlying cause and should be included in the same cohort; that cases with any parkinsonism features should be excluded to improve phenotype and presumably genotype homogeneity; that ET–parkinsonism cases are an informative subset with distinct causal mechanism(s).

A related approach is to use phenotype variables instead of disease diagnosis as the outcome against genetic predictors. For example, a genetic variant could be tested against a tremor feature, within ET or regardless of ET or PD clinical diagnosis. As an illustration of principle, asymmetric tremor severity is often observed in PD, although not all PD cases have any tremor. The ET clinical definition includes both upper extremity tremor in isolation, and head tremor in isolation.[18,115,128] ET versus PD genetic papers rarely comment on whether PD cases were tremor predominant, or if ET cases include those with head tremor alone. Current clinical definitions stress bilateral upper extremity tremor in ET with unilateral tremor as a red flag indicating an alternative diagnosis, but tremor severity in ET may be asymmetric,[24,129] and many descriptions of ET note frequent unilateral onset with eventual (2–3 years) spread to both upper extremities.[10,19,23,25] Asymmetric severity and unilateral tremor onset may predict greater ET progression.[130] Asymmetric tremor severity, or even just the presence of upper extremity tremor, can be tested against genotypes within or between ET and PD cohorts. As above, outward tremor features may or may not indicate underlying mechanistic connections: when hypotheses are structured around phenotype variables instead of diagnoses, the door is open to either outcome.

Non-tremor features of ET are also rarely explored in genetics research. Clinical scales focus on tremor, often biased to upper extremity kinetic tremor. Mild imbalance manifesting as impaired tandem gait is common in ET.[131–134] ET subjects have significant impairments on multiple measures of postural control and functional mobility, independent of tremor body area (head or no head tremor) and tremor severity.[135] Dystonia as an ET motor feature is considered below. Non-motor features such as mood and cognitive changes are under ongoing study but are not considered a clear part of clinically defined ET.[136] Genetic risks could be tested against any of these outcomes, again within ET or across ET and other clinically defined groups. This adds both richer information and more flexibility to research designs. Major challenges to this approach are agreeing on phenotype variables across research groups, having sufficient power to address multiple testing issues, and addressing subject burden as research participation time increases. Regarding agreement on phenotype datasets, testing hypotheses in this type of structure does not commit the clinical ET definition to change, or imply that phenotype variables are clearly part of ET. It improves the ability to explore a range of possible outcomes, whether ET and other disorders share mechanistic connections or not—or whether phenotype-variable subsets do a better job representing genotypes than diagnosis subsets, or not.

Dystonia and ET is another contested area of potential mechanistic connection, with some genetics data behind it. Concurrent dystonia is an exclusion criterion in the current ET clinical diagnosis.[18,115,128] On the other hand, head tremor in isolation is considered clinical ET, but cervical dystonia often causes head tremor. As above, ET genetics studies rarely record whether ET cases include head tremor, arm tremor, or both; nor do they generally specify if dystonia was specifically queried or examined. Dystonia is therefore a source of ET misdiagnoses, i.e., false positives. More interesting is considering dystonia as a potentially useful phenotype variable. Tremor and dystonia can occur in separate body areas. A relevant example is ET-like arm tremor with cervical dystonia, a phenotype of highly contested classification (see Schiebler et al[137] for review). Dystonia in a non-tremor area may develop long after the tremor, reinforcing how longitudinal information about disease course is important in forming ET subsets.[137] Outside of the ET clinical definition debate, there is movement towards recognizing (by any label) ET and dystonia, or recording specific variables such as arm tremor, head/neck tremor, and cervical dystonia. These arguments parallel the PD and tremor examples above. For dystonia, motivation to change ET research approaches also comes from genetics.

Phenotypes can be considered at the pedigree as well as the individual case level. In the ETM3 linkage study, dystonia was not only specifically recorded for all family members; the 6p23 locus was only linked in families with a mix of dystonia and ET.[36] A detailed ET phenotype study utilizing in-person movement disorders examinations

of all probands and family members observed multiple cases of dystonia, with or without ET in each individual case, in 28% of 97 ET kindreds.[138] This provides further motivation for an ET–dystonia subset. As in ET–PD or ET–parkinsonism, the hypothesis is that pedigree-level ET subsets can reflect underlying genotypes in some cases.

What if kinetic arm tremor plus cervical dystonia isn't "real" ET? We do not know what real ET will be in a future, more causal mechanism-driven definition set. Currently we assume ET clinical definitions match underlying disease mechanisms. Any ET research phenotype choice could increase rather than decrease genetic heterogeneity compared with an ET clinical diagnosis group. Conversely, one genotype may cause a wide range of phenotypes (Tables 2 and 3). This challenge can be experienced as a stalemate, where any phenotype choice is equally wrong, as we do not yet know phenotype–genotype connections. Instead, using a rich, open phenotyping approach broadens the range of testable hypotheses, including whether or not a given phenotype is related to a genotype, or ultimately to a clinical diagnosis. A shift from binary ET diagnosis outcome based on limited clinical criteria to research-oriented phenotypes generates progress by opening up possibilities in genetics experiment design.

The conceptual shift in ET phenotyping is reflected in research techniques. In addition to more videotaped detailed examination data, and use of validated scales across tremor and non-tremor features, investigators are incorporating objective measures such as digitized spiral analysis, electromyography, and accelerometry recording (see Shatunov et al[36] for example). Calls for longitudinal detailed ET phenotype cohort studies with neuropathological follow-up are well founded:[117,139] such studies would greatly enhance ET genetics work by providing the level of phenotype data needed, tied to neuropathological diagnosis as well as clinical diagnosis. Phenotypically homogeneous criteria are driven clinically, not by biomarkers or other reliable gold standards. Neuropathology is the gold standard diagnosis for PD, although not ET or dystonia; still this is a way to greatly enhance data used for decision making in genetic sample analyses.

Improving ET phenotyping includes basic issues such as direct examination of research subjects. Redefining ET phenotyping encompasses incorporating a range of motor, non-motor, examination, objective measure, prospective, longitudinal, and neuropathological data into a rich, fruitful resource. New approaches to ET phenotyping will enable ET research to fully exploit advancing genetics technologies.

From the genetics side: mechanism of inheritance

Working within a genetic framework, the ET field is already moving into new ways to detect and analyze patterns of disease-associated change. ET was originally conceptualized as a phenotypically homogeneous familial entity, caused by common genetic variants. ET is better characterized as a complex trait;[14,121] pathophysiology could therefore involve rare genetic variants in combination, instead of a few common variants.[121,140–143] As reflected by the association studies in ET (Table 1), the field is moving from single genetic variant analysis to more detailed, comprehensive sequencing approaches of candidate areas or the genome. While ET genetics thinking has already started to shift, integrating new phenotyping approaches and use of different phenotyping datasets against genotype data remain rare. This section outlines key ET challenges from the genetics side: whether and how to distinguish familial and sporadic ET; ET mechanisms of inheritance; and moving into new genetics technologies.

Genetic research reports often distinguish between "familial" and "sporadic" ET, even though this distinction is considered supporting not primary data for clinical definition,[18] and is not currently clinically useful. Is the assumption that ET can be divided into familial and sporadic cases useful for research? A mix of familial and sporadic disease in a cohort could certainly affect attempts at genetics research: *LINGO1* results may be stronger in familial ET.[14] However, it is often unclear what these terms mean in ET. There is little consensus on whether studies should focus in on subjects with an extensive, clear family history, or not—or what constitutes extensive or clear. Many issues make ET family history challenging to interpret: "senile tremor" is dismissed by patients and providers, mild tremor may be unknown to other family members, ET is often misdiagnosed, direct examinations of all family members may be appropriate but not feasible. Given these issues plus the tremendous percentage of positive family history in studies discussed above, one valid approach is to disregard sporadic and familial subsets as not useful at this time.[26] An alternative is to rank the quality of family history, where a conservative definition of positive family history may hinder acquiring large sample sizes and exclude informative cases, but improve overall data quality and help focus genetics work on a potentially powerful ET subset.

Even with the above caveats, the well-reported high rate of positive family history is an obvious starting point for ET research. There is varied and convincing evidence that much of ET is inherited in an autosomal dominant fashion,[45,144] although complex multigenetic modes of inheritance cannot yet be excluded.[22,36,45,116,145] The field has already moved into deep sequencing both coding and non-coding DNA stretches, or utilizing full exome strategies: in the first case with mixed results, and in the latter with conspicuously silent results. This could reasonably be blamed on the unfortunate wealth of inherent ET phenotype issues discussed above (Table 2), as well as natural shaking out of replication attempts from early smaller studies. Another factor may be a main feature of ET: assumptions based on pedigree appearances of a highly prevalent and penetrant autosomal dominant disorder (Table 3).

The high number of affected individuals in ET families was initially attractive to research groups. The high prevalence and high rate of affected family members, while potentially challenging for genetics studies, were not considered an indication of primary mechanism.[26] For example, the ET parish study concluded that chance variations in previous generations when the parish population was very small were enough to account for the high phenotype and thus assumed high genetic variant frequency[25]. As more and more familial linkage studies

returned negative results, some suspected "too many" affecteds for a straightforward autosomal dominant mechanism.[145] A combination of phenocopies, ascertainment bias, and non-ET tremors (physiologic tremor, PD, medications) could explain the high reported affected numbers, as above. A non-Mendelian inheritance pattern presents an alternate explanation.[98]

Epigenetics encompasses mechanisms that change gene expression or activity without changing DNA sequences: DNA methylation and histone modification are classic examples. Epigenetic states can, at least in part, be inherited.[146] This type of inheritance can occur in humans, through unclear mechanisms (reviewed in Zimprich[98]). Epigenetic variation would not be detected by genome sequencing experiments. In a recent hypothesis paper, Zimprich details how epigenetic inheritance could explain observations in ET.[98] An epigenetic feature may be transmitted from one allele to another in a parent cell prior to meiosis; thus in the gametes both the disease-causing allele and the originally benign allele become disease causing. This is termed paramutation.[147] Long described in plants, the role of paramutation in mammals, particularly humans, is postulated from observations in diabetes mellitus but is uncertain.[98,148] Zimprich puts forward a framework of primarily epigenetic and paramutation inheritance, rather than genetic state, accounting for ET pedigree phenotype patterns.[98] This intriguing theory pushes the field to consider diverse mechanism options. Pursuing this theory will require advances in detecting and analyzing epigenetic changes; for example, "methylome" analysis.[149]

Another alternate explanation of ET inheritance remains within genetics: ET may be a complex trait, with work to date identifying only a limited amount of the heritable component of ET.[15,22,36,45,116,121,145] Uncovering "missing heritability" in ET may require researchers to pursue rare rather than common causal genetics variants, and/or many genetic risk factors.[15,121,140] As in epigenetics, progress in genetics is often made through advancing technologies. Next-generation sequencing allows sequencing the whole genome, for example from members of one pedigree in an attempt to identify a causal mutation. High-throughput sequencing can be restricted to exons (exome sequencing).[150] New sequencing technology holds great promise for testing the theory that ET is a complex trait caused by combinations of rare genetic variants, rather than a family of disorders caused by a small number of common causal mutations. Experiments utilizing next generation sequencing to achieve detailed analysis across coding, intronic, and regulatory areas could help detect rare genetic variants contributing to ET.[15,121,140–142]

Advances in analyzing large datasets will also be crucial. This can be considered across a spectrum of genetic data: rare variants with a high impact on disease causation, low-frequency genetic variants with intermediate effects, and combinations of relatively common variants acting as genetic risk factors each with a small effect size.[140,151] Already, meta-analyses leveraging multiple independent GWAS datasets in PD have set an example for detecting genetic risk factors with small effect sizes.[151] Considering genetic and environmental risk factor data together is another potentially fruitful approach[152] that demands advances in large dataset analyses.

New sequencing technologies are also necessary for detecting the full range of genetic variants that may contribute to ET. Structure variants are not single nucleotide (SNP) changes; instead the term encompasses various changes such as insertions, deletions, and DNA sequence inversions.[140] One structural variant form is copy number variants: DNA stretches that are usually unique are repeated, in duplicate or triplicate. Their size in base pairs varies widely. Efforts focusing on specific genetic regions have uncovered copy number variant effects in movement disorders such as PD and chorea–acanthocytosis.[153,154] New array technologies are greatly expanding the ability to detect copy number variants.[155] Detecting copy number and other structural variants on a large scale is hampered by sequencing technology limitations, cost, statistical power issues, and challenges in interpreting the clinical significance of observed variants.[140,155]

Genetic approaches will benefit from lowering costs and advances in epigenetic methods, high-throughput sequencing, and high-dimensional data analysis. For ET genetics research, progress will come as much from evolving phenotype work as new "-omics". Contributions from both the genetics and the movement disorders sides position the field to meet ET research challenges.

Moving forward

Collaborators in movement disorders and genetics are rethinking fundamental assumptions and experimental designs to better advance knowledge in the field (Table 3). This includes using a range of phenotype outcomes from clinical diagnosis labels and ET subsets to phenotype data points within or across ET, PD, and other disorders. Gathering detailed prospective longitudinal phenotype data instead of clinical opinion on diagnosis or retrospective record review represents a significant shift in culture. Video-recording examinations, even if the in-person data are the outcomes, so the primary data are the actual examinations not the opinion of the examiner, is becoming standard; whether video rating rather than in-person rating is sufficient for all studies is an open question. In rethinking genetic approaches, the field is already moving on: examples include efforts at expanding collaborations[36,49,91]; considering intronic[46,90,91] and regulatory[90,91] as well as coding regions; utilizing a variety of linkage and association analysis techniques.[36,49,51,91] To some extent, genetics efforts are also moving on from older phenotype strategies: for example, using definite ET for affected status,[16,36,48,90,91] and including data on dystonia.[36] Overall, investigators are considering how to test novel hypotheses to account for observations in ET: how to detect rare genetic variants contributing to a complex disorder, or epigenetic changes contributing to different modes of inheritance; how to test different phenotype structures against genotypes.

The biggest shift may be moving from debates about phenotype or genotype in isolation to how to attack the interactions between the two. In ET genetics research, the days of handing over or accepting an isolated set of de-identified samples labeled ET or control are over. Collaboration now means considering both phenotype and genetic

technique choices together, with input from the movement disorders and genetics sides. Moving beyond assumptions in the field creates new challenges; for example, finding a balance between communicating about experiment design before samples and phenotype data are collected, and allowing flexibility for advances in genetics technology, data analysis, and phenotype definitions over time. As in many slowly progressive later onset disorders, a central balancing act is between cost versus phenotype data detail, range of prospective data time points, types of genetics sample processing, and ability to analyze high dimensional data. We can take encouragement from successful examples in Huntington disease[156] and PD[157–159] when advocating for the level of ET phenotype–genotype work necessary to create scientific advances in the field.

Some issues, like ethical and pragmatic consideration in sharing biosamples, affect genetics work in many areas. Given results to date, working through these hurdles represents an acute challenge in ET.[121] The most recent locus, ETM3, represented work across several groups;[36] the GWAS required input and multiple confirmatory cohorts from collaborators.[46] Of the association studies reviewed in Table 1, the only ones above an ET n of 500[91] or 1000[49] were from the start of a North American Essential Tremor Consortium collaborative effort in ET genetics, which grew out of the TRIG and now includes over 14 groups. We hope to contribute more results across many more consortium members, and encourage other groups to do the same.

These kinds of studies are necessarily collaborative. A key element in these collaborations is the research subject community (Table 3). Patients and families volunteer their time, information about themselves and family members, and biosamples. ET patients and families are often highly motivated to participate in genetics research, and are beginning to organize from a research participant perspective. How and when to return genetic research data to subjects and families is a general topic in genetics,[155,160] and as yet unexplored in ET. How to effectively include participation from subjects with a range of ethnic backgrounds in the context of rigorous genetics design, particularly in North American work, is also an underdeveloped area.

Conclusions

In summary, lack of an ET biomarker is a challenge to the research community, not a stalemate. Increased understanding of ET genetics will be an important step in ET biomarker development. Large collaborative efforts including genetics and movement disorders expertise are forming. We are only beginning to engage the patient and family community central to the research on a collaborative level. The ability for multiple groups to generate and analyze detailed genetic data under varied experimental models is increasing: work is still critically needed on minimum phenotyping datasets that can be consistent across many groups in prospective studies, but at the same time are well beyond a binary clinical diagnosis. In the end, we may experience a major shift in ET and beyond as we uncover mechanisms behind movement disorders manifestations: "causal factors of disease, when identified, not uncommonly have effects that cross the boundaries of adjacent manifestational groups … Change from a manifestational to a causal axis of classification may result in a major regrouping of impaired individuals."[114] Despite the hurdles, it is an exciting time in the field, when researchers have the opportunity to find new ways to work together towards progress in an important and wide-open area.

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References

1. Dogu O, Louis ED, Sevim S, Kaleagasi H, Aral M. Clinical characteristics of essential tremor in Mersin, Turkey: A population-based door-to-door study. *J Neurol* 2005;252:570–574, doi: http://dx.doi.org/10.1007/s00415-005-0700-8.

2. Rajput A, Robinson CA, Rajput AH. Essential tremor course and disability: A clinicopathologic study of 20 cases. *Neurology* 2004;62:932–936, doi: http://dx.doi.org/10.1212/01.WNL.0000115145.18830.1A.

3. Louis ED, Barnes L, Albert SM, et al. Correlates of functional disability in essential tremor. *Mov Disord* 2001;16:914–920, doi: http://dx.doi.org/10.1002/mds.1184.

4. Sullivan KL, Hauser RA, Zesiewicz TA. Essential tremor. Epidemiology, diagnosis, and treatment. *Neurologist* 2004;10:250–258, doi: http://dx.doi.org/10.1097/01.nrl.0000138736.07840.b2.

5. Louis ED, Ferreira JJ. How common is the most common adult movement disorder? Update on the worldwide prevalence of essential tremor. *Mov Disord* 2010;25:534–541, doi: http://dx.doi.org/10.1002/mds.22838.

6. Dogu O, Sevim S, Camdeviren H, et al. Prevalence of essential tremor: door-to-door neurologic exams in Mersin Province, Turkey. *Neurology* 2003;61:1804–1806, doi: http://dx.doi.org/10.1212/01.WNL.0000099075.19951.8C.

7. Elble RJ. Tremor in ostensibly normal elderly people. *Mov Disord* 1998;13:457–464, doi: http://dx.doi.org/10.1002/mds.870130314.

8. Tanner CM, Goldman SM. Epidemiology of Parkinson's disease. *Neurol Clin* 1996;14:317–335, doi: http://dx.doi.org/10.1016/S0733-8619(05)70259-0.

9. Lyons KE, Pahwa R, Comella CL, et al. Benefits and risks of pharmacological treatments for essential tremor. *Drug Saf* 2003;26:461–481, doi: http://dx.doi.org/10.2165/00002018-200326070-00003.

10. Critchley M. Observations on essential (heredofamial) tremor. *Brain* 1949;72:113–139, doi: http://dx.doi.org/10.1093/brain/72.2.113.

11. Louis ED, Broussolle E, Goetz CG, Krack P, Kaufmann P, Mazzoni P. Historical underpinnings of the term essential tremor in the late 19th century. *Neurology* 2008;71:856–859, doi: http://dx.doi.org/10.1212/01.wnl.0000325564.38165.d1.

12. Dana CL. Hereditary tremor, a hitherto undescribed form of motor neurosis. *Am J Med Sci* 1887;94:386–393, doi: http://dx.doi.org/10.1097/00000441-188710000-00005.

13. Tan EK. LINGO1 and essential tremor: linking the shakes. Linking LINGO1 to essential tremor. *Eur J Hum Genet* 2010;18:739–740, doi: http://dx.doi.org/10.1038/ejhg.2010.25.

14. Jasinska-Myga B, Wider C. Genetics of essential tremor. *Parkinsonism Relat Disord* 2012;18:S138–139, doi: http://dx.doi.org/10.1016/S1353-8020(11)70043-8.

15. Zimprich A. Genetics of Parkinson's disease and essential tremor. *Curr Opin Neurol* 2011;24:318–323, doi: http://dx.doi.org/10.1097/WCO.0b013e3283484b87.

16. Lorenz D, Frederiksen H, Moises H, Kopper F, Deuschl G, Christensen K. High concordance for essential tremor in monozygotic twins of old age. *Neurology* 2004;62:208–211, doi: http://dx.doi.org/10.1212/01.WNL.0000103236.26934.41.

17. Tanner CM, Goldman SM, Lyons KE, et al. Essential tremor in twins: an assessment of genetic vs environmental determinants of etiology. *Neurology* 2001;57:1389–1391, doi: http://dx.doi.org/10.1212/WNL.57.8.1389.

18. Deuschl G, Bain P, Brin M, and the Ad Hoc Scientific Committee. Consensus statement of the Movement Disorder Society on Tremor. *Mov Disord* 1998;13:2–23, doi: http://dx.doi.org/10.1002/mds.870131303.

19. Whaley NR, Putzke JD, Baba Y, Wszolek ZK, Uitti RJ. Essential tremor: phenotypic expression in a clinical cohort. *Parkinsonism Relat Disord* 2007;13:333–339, doi: http://dx.doi.org/10.1016/j.parkreldis.2006.12.004.

20. Koller WC, Busenbark K, Miner K. The relationship of essential tremor to other movement disorders: report on 678 patients. Essential Tremor Study Group. *Ann Neurol* 1994;35:717–723, doi: http://dx.doi.org/10.1002/ana.410350613.

21. Lou JS, Jankovic J. Essential tremor: clinical correlates in 350 patients. *Neurology* 1991;41:234–238, doi: http://dx.doi.org/10.1212/WNL.41.2_Part_1.234.

22. Louis ED, Ottman R. How familial is familial tremor? The genetic epidemiology of essential tremor. *Neurology* 1996;46:1200–1205, doi: http://dx.doi.org/10.1212/WNL.46.5.1200.

23. Critchley E. Clinical manifestations of essential tremor. *J Neurol Neurosurg Psychiatry* 1972;35:365–372, doi: http://dx.doi.org/10.1136/jnnp.35.3.365.

24. Dotchin CL, Walker RW. The prevalence of essential tremor in rural northern Tanzania. *J Neurol Neurosurg Psychiatry* 2008;79:1107–1109, doi: http://dx.doi.org/10.1136/jnnp.2007.134304.

25. Larsson T, Sjogren T. Essential tremor: a clinical and genetic population study. *Acta Psychiatr Scand Suppl* 1960;36:1–176.

26. Busenbark K, Barnes P, Lyons K, Ince D, Villagra F, Koller WC. Accuracy of reported family histories of essential tremor. *Neurology* 1996;47:264–265, doi: http://dx.doi.org/10.1212/WNL.47.1.264.

27. Louis ED. Essential tremor. *Lancet Neurol* 2005;4:100–110, doi: http://dx.doi.org/10.1016/S1474-4422(05)00991-9.

28. Elble RJ. Diagnostic criteria for essential tremor and differential diagnosis. *Neurology* 2000;54:S2–6, doi: http://dx.doi.org/10.1212/WNL.54.1.2.

29. Findley LJ. Epidemiology and genetics of essential tremor. *Neurology* 2000;54:S8–S13, doi: http://dx.doi.org/10.1212/WNL.54.1.8.

30. Morton NE. Sequential tests for the detection of linkage. *Am J Hum Genet* 1955;7:277–318.

31. Gulcher JR, Jonsson P, Kong A, et al. Mapping of a familial essential tremor gene, FET1, to chromosome 3q13. *Nat Genet* 1997;17:84–87, doi: http://dx.doi.org/10.1038/ng0997-84.

32. Illarioshkin SN, Rakhmonov RA, Ivanova-Smolenskaia IA, et al. Molecular genetic analysis of essential tremor. *Genetika* 2002;38:1704–1709.

33. Higgins JJ, Pho LT, Nee LE. A gene (ETM) for essential tremor maps to chromosome 2p22–p25. *Mov Disord* 1997;12:859–864, doi: http://dx.doi.org/10.1002/mds.870120605.

34. Higgins JJ, Loveless JM, Jankovic J, Patel PI. Evidence that a gene for essential tremor maps to chromosome 2p in four families. *Mov Disord* 1998;13:972–977, doi: http://dx.doi.org/10.1002/mds.870130621.

35. Higgins JJ, Lombardi RQ, Pucilowska J, Ruszczyk MU. Integrated physical map of the human essential tremor gene region (ETM2) on chromosome 2p24.3–p24.2. *Am J Med Genet B Neuropsychiatr Genet* 2004;127:128–130, doi: http://dx.doi.org/10.1002/ajmg.b.20152.

36. Shatunov A, Sambuughin N, Jankovic J, et al. Genomewide scans in North American families reveal genetic linkage of essential tremor to a region on chromosome 6p23. *Brain* 2006;129:2318–2331, doi: http://dx.doi.org/10.1093/brain/awl120.

37. Kim JH, Cho YH, Kim JK, Park YG, Chang JW. Frequent sequence variation at the ETM2 locus and its association with sporadic essential tremor in Korea. *Mov Disord* 2005;20:1650–1653, doi: http://dx.doi.org/10.1002/mds.20646.

38. Zahorakova D, Ulmanova O, Kemlink D, et al. No association with the ETM2 locus in Czech patients with familial essential tremor. *Neuro Endocrinol Lett* 2010;31:549–552.

39. Inashkina I, Radovica I, Smeltere L, Vitols E, Jankevics E. Case-control study of patients with essential tremor in Latvia. *Eur J Neurol* 2008;15:988–990, doi: http://dx.doi.org/10.1111/j.1468-1331.2008.02225.x.

40. Novelletto A, Gulli R, Ciotti P, et al. Linkage exclusion in Italian families with hereditary essential tremor. *Eur J Neurol* 2011;18:e118–120, doi: http://dx.doi.org/10.1111/j.1468-1331.2011.03458.x.

41. Aridon P, Ragonese P, De Fusco M, Salemi G, Casari G, Savettieri G. Further evidence of genetic heterogeneity in familial essential tremor. *Parkinsonism Relat Disord* 2008;14:15–18, doi: http://dx.doi.org/10.1016/j.parkreldis.2007.05.002.

42. Illarioshkin SN, Ivanova-Smolenskaya IA, Rahmonov RA, Markova ED, Stevanin G, Brice A. Clinical and genetic study of familial essential tremor in an isolate of Northern Tajikistan. *Mov Disord* 2000;15:1020–1023, doi: http://dx.doi.org/10.1002/1531-8257(200009)15:5<1020::AID-MDS1044>3.0.CO;2-G.

43. Abbruzzese G, Pigullo S, Di Maria E, et al. Clinical and genetic study of essential tremor in the Italian population. *Neurol Sci* 2001;22:39–40, doi: http://dx.doi.org/10.1007/s100720170036.

44. Kovach MJ, Ruiz J, Kimonis K, et al. Genetic heterogeneity in autosomal dominant essential tremor. *Genet Med* 2001;3:197–199, doi: http://dx.doi.org/10.1097/00125817-200105000-00009.

45. Deng H, Le W, Jankovic J. Genetics of essential tremor. *Brain* 2007;130:1456–1464, doi: http://dx.doi.org/10.1093/brain/awm018.

46. Stefansson H, Steinberg S, Petursson H, et al. Variant in the sequence of the LINGO1 gene confers risk of essential tremor. *Nat Genet* 2009;41:277–279, doi: http://dx.doi.org/10.1038/ng.299.

47. Deng H, Gu S, and Jankovic J. LINGO1 variants in essential tremor and Parkinson's disease. *Acta Neurol Scand* 2012;125:1–7, doi: http://dx.doi.org/10.1111/j.1600-0404.2011.01516.x.

48. Thier S, Lorenz D, Nothnagel M, et al. LINGO1 polymorphisms are associated with essential tremor in Europeans. *Mov Disord* 2010;25:709–715, doi: http://dx.doi.org/10.1002/mds.22887.

49. Vilarino-Guell C, Wider C, Ross OA, et al. LINGO1 and LINGO2 variants are associated with essential tremor and Parkinson disease. *Neurogenetics* 2010;11:401–408, doi: http://dx.doi.org/10.1007/s10048-010-0241-x.

50. Vilarino-Guell C, Ross OA, Wider C, et al. LINGO1 rs9652490 is associated with essential tremor and Parkinson disease. *Parkinsonism Relat Disord* 2010;16:109–111, doi: http://dx.doi.org/10.1016/j.parkreldis.2009.08.006.

51. Clark LN, Park N, Kisselev S, Rios E, Lee JH, Louis ED. Replication of the LINGO1 gene association with essential tremor in a North American population. *Eur J Hum Genet* 2010;18:838–843, doi: http://dx.doi.org/10.1038/ejhg.2010.27.

52. Radovica I, Inashkina I, Smeltere L, Vitols E, Jankevics E. Screening of 10 SNPs of LINGO1 gene in patients with essential tremor in the Latvian population. *Parkinsonism Relat Disord* 2012;18:93–95, doi: http://dx.doi.org/10.1016/j.parkreldis.2011.06.006.

53. Tan EK, Teo YY, Prakash KM, et al. LINGO1 variant increases risk of familial essential tremor. *Neurology* 2009;73:1161–1162, doi: http://dx.doi.org/10.1212/WNL.0b013e3181bacfc9.

54. Zuo X, Jiang H, Guo JF, et al. Screening for two SNPs of LINGO1 gene in patients with essential tremor or sporadic Parkinson's disease in Chinese population. *Neurosci Lett* 2010;481:69–72, doi: http://dx.doi.org/10.1016/j.neulet.2010.06.041.

55. Lorenzo-Betancor O, Garcia-Martin E, Cervantes S, et al. Lack of association of LINGO1 rs9652490 and rs11856808 SNPs with familial essential tremor. *Eur J Neurol* 2011;18:1085–1089, doi: http://dx.doi.org/10.1111/j.1468-1331.2010.03251.x.

56. Bourassa CV, Riviere JB, Dion PA, et al. LINGO1 variants in the French-Canadian population. *PLoS One* 2011;6:e16254, doi: http://dx.doi.org/10.1371/journal.pone.0016254.

57. Wu YW, Rong TY, Li HH, et al. Analysis of Lingo1 variant in sporadic and familial essential tremor among Asians. *Acta Neurol Scand* 2011;124:264–268, doi: http://dx.doi.org/10.1111/j.1600-0404.2010.01466.x.

58. Wu YW, Prakash KM, Rong TY, et al. Lingo2 variants associated with essential tremor and Parkinson's disease. *Hum Genet* 2011;129:611–615, doi: http://dx.doi.org/10.1007/s00439-011-0955-3.

59. Liang H, Zheng W, Xu H, et al. No evidence of association between the LINGO4 gene and essential tremor in Chinese Han patients. *Parkinsonism Relat Disord* 2012;18:303–305, doi: http://dx.doi.org/10.1016/j.parkreldis.2011.10.017.

60. Higgins JJ, Lombardi RQ, Pucilowska J, Jankovic J, Tan EK, Rooney JP. A variant in the HS1-BP3 gene is associated with familial essential tremor. *Neurology* 2005;64:417–421, doi: http://dx.doi.org/10.1212/01.WNL.0000153481.30222.38.

61. Higgins JJ, Lombardi RQ, Pucilowska J, Jankovic J, Golbe LI, Verhagen L. HS1-BP3 gene variant is common in familial essential tremor. *Mov Disord* 2006;21:306–309, doi: http://dx.doi.org/10.1002/mds.20692.

62. Deng H, Le WD, Guo Y, Huang MS, Xie WJ, Jankovic J. Extended study of A265G variant of HS1BP3 in essential tremor and Parkinson disease. *Neurology* 2005;65:651–652, doi: http://dx.doi.org/10.1212/01.wnl.0000173033.32535.23.

63. Shatunov A, Jankovic J, Elble R, et al. A variant in the HS1-BP3 gene is associated with familial essential tremor. *Neurology* 2005;65:1995; with author reply 1995, doi: http://dx.doi.org/10.1212/01.wnl.0000200984.10076.e5.

64. Testa CM, Miyatake L, Wilson M, Bouzyk M, Factor S. Longer REP1 repeat lengths are associated with both essential tremor (ET) and Parkinson disease (PD). *Mov Disord* 2008;23:S35, doi: http://dx.doi.org/10.1002/mds.21727.

65. Jeanneteau F, Funalot B, Jankovic J, et al. A functional variant of the dopamine D3 receptor is associated with risk and age-at-onset of essential tremor. *Proc Natl Acad Sci USA* 2006;103:10753–10758, doi: http://dx.doi.org/10.1073/pnas.0508189103.

66. Garcia-Martin E, Martinez C, Alonso-Navarro H, et al. Dopamine receptor D3 (DRD3) genotype and allelic variants and risk for essential tremor. *Mov Disord* 2009;24:1910–1915, doi: http://dx.doi.org/10.1002/mds.22518.

67. Blair MA, Ma S, Phibbs F, et al. Reappraisal of the role of the DRD3 gene in essential tremor. *Parkinsonism Relat Disord* 2008.

68. Tan EK, Prakash KM, Fook-Chong S, et al. DRD3 variant and risk of essential tremor. *Neurology* 2007;68:790–791, doi: http://dx.doi.org/10.1212/01.wnl.0000256773.87498.2f.

69. Lorenz D, Klebe S, Stevanin G, et al. Dopamine receptor D3 gene and essential tremor in large series of German, Danish and French patients. *Eur J Hum Genet* 2009;17:766–773, doi: http://dx.doi.org/10.1038/ejhg.2008.243.

70. Vitale C, Gulli R, Ciotti P, et al. DRD3 Ser9Gly variant is not associated with essential tremor in a series of Italian patients. *Eur J Neurol* 2008;15:985–987, doi: http://dx.doi.org/10.1111/j.1468-1331.2008.02164.x.

71. Testa CM. Tremor, essential (syndromes). In: Kompoliti K, Verhagen L(eds.) *The Encyclopedia of Movement Disorders*. 2010: Elsevier and Science Direct (ebook).

72. Vilarino-Guell C, Soto-Ortolaza AI, Rajput A, et al. MAPT H1 haplotype is a risk factor for essential tremor and multiple system atrophy. *Neurology* 2011;76:670–672, doi: http://dx.doi.org/10.1212/WNL.0b013e31820c30c1.

73. Clark LN, Kisselev S, Park N, et al. Mutations in the Parkinson's disease genes, Leucine Rich Repeat Kinase 2 (LRRK2) and Glucocerebrosidase (GBA), are not associated with essential tremor. *Parkinsonism Relat Disord* 2010;16:132–135, doi: http://dx.doi.org/10.1016/j.parkreldis.2009.05.008.

74. Vitale C, Ciotti P, Gulli R, et al. Common mutations in the LRRK2 exon 41 are not responsible for essential tremor in Italian patients. *Parkinsonism Relat Disord* 2009;15:162–163, doi: http://dx.doi.org/10.1016/j.parkreldis.2008.04.035.

75. Tan EK, Matsuura T, Nagamitsu S, Khajavi M, Jankovic J, and Ashizawa T. Polymorphism of NACP-Rep1 in Parkinson's disease: an etiologic link with essential tremor? *Neurology* 2000;54:1195–1198, doi: http://dx.doi.org/10.1212/WNL.54.5.1195.

76. Polymeropoulos MH, Lavedan C, Leroy E, et al. Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science* 1997;276:2045–2047, doi: http://dx.doi.org/10.1126/science.276.5321.2045.

77. Kruger R, Kuhn W, Muller T, et al. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat Genet* 1998;18:106–108, doi: http://dx.doi.org/10.1038/ng0298-106.

78. Zarranz JJ, Alegre J, Gomez-Esteban JC, et al. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Ann Neurol* 2004;55:164–173, doi: http://dx.doi.org/10.1002/ana.10795.

79. Singleton AB, Farrer M, Johnson J, et al. Alpha-synuclein locus triplication causes Parkinson's disease. *Science* 2003;302:841, doi: http://dx.doi.org/10.1126/science.1090278.

80. Farrer M, Kachergus J, Forno L, et al. Comparison of kindreds with parkinsonism and alpha-synuclein genomic multiplications. *Ann Neurol* 2004;55:174–179, doi: http://dx.doi.org/10.1002/ana.10846.

81. Chartier-Harlin MC, Kachergus J, Roumier C, et al. Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet* 2004;364:1167–1169, doi: http://dx.doi.org/10.1016/S0140-6736(04)17103-1.

82. Ibanez P, Bonnet AM, Debarges B, et al. Causal relation between alpha-synuclein gene duplication and familial Parkinson's disease. *Lancet* 2004;364:1169–1171, doi: http://dx.doi.org/10.1016/S0140-6736(04)17104-3.

83. Mellick GD, Maraganore DM, Silburn PA. Australian data and meta-analysis lend support for alpha-synuclein (NACP-Rep1) as a risk factor for Parkinson's disease. *Neurosci Lett* 2005;375:112–116, doi: http://dx.doi.org/10.1016/j.neulet.2004.10.078.

84. Maraganore DM, de Andrade M, Elbaz A, et al. Collaborative analysis of alpha-synuclein gene promoter variability and Parkinson disease. *JAMA* 2006;296:661–670, doi: http://dx.doi.org/10.1001/jama.296.6.661.

85. Kruger R, Vieira-Saecker AM, Kuhn W, et al. Increased susceptibility to sporadic Parkinson's disease by a certain combined alpha-synuclein/apolipoprotein E genotype. *Ann Neurol* 1999;45:611–617, doi: http://dx.doi.org/10.1002/1531-8249(199905)45:5<611::AID-ANA9>3.0.CO;2-X.

86. Farrer M, Maraganore DM, Lockhart P, et al. alpha-Synuclein gene haplotypes are associated with Parkinson's disease. *Hum Mol Genet* 2001;10:1847–1851, doi: http://dx.doi.org/10.1093/hmg/10.17.1847.

87. Pals P, Lincoln S, Manning J, et al. alpha-Synuclein promoter confers susceptibility to Parkinson's disease. *Ann Neurol* 2004;56:591–595, doi: http://dx.doi.org/10.1002/ana.20268.

88. Hadjigeorgiou GM, Xiromerisiou G, Gourbali V, et al. Association of alpha-synuclein Rep1 polymorphism and Parkinson's disease: influence of Rep1 on age at onset. *Mov Disord* 2006;21:534–539, doi: http://dx.doi.org/10.1002/mds.20752.

89. Kay DM, Zabetian CP, Factor SA, et al. Parkinson's disease and LRRK2: frequency of a common mutation in U.S. movement disorder clinics. *Mov Disord* 2006;21:519–523, doi: http://dx.doi.org/10.1002/mds.20751.

90. Pigullo S, Di Maria E, Marchese R, et al. Essential tremor is not associated with alpha-synuclein gene haplotypes. *Mov Disord* 2003;18:823–826, doi: http://dx.doi.org/10.1002/mds.10421.

91. Ross OA, Conneely KN, Wang T, et al. Genetic variants of alpha-synuclein are not associated with essential tremor. *Mov Disord* 2011;26:2552–2556, doi: http://dx.doi.org/10.1002/mds.23909.

92. Kralic JE, Criswell HE, Osterman JL, et al. Genetic essential tremor in gamma-aminobutyric acidA receptor alpha1 subunit knockout mice. *J Clin Invest* 2005;115:774–779.

93. Thier S, Kuhlenbaumer G, Lorenz D, et al. GABA(A) receptor- and GABA transporter polymorphisms and risk for essential tremor. *Eur J Neurol* 2011;18:1098–1100, doi: http://dx.doi.org/10.1111/j.1468-1331.2010.03308.x.

94. Garcia-Martin E, Martinez C, Alonso-Navarro H, et al. Gamma-aminobutyric acid (GABA) receptor rho (GABRR) polymorphisms and risk for essential tremor. *J Neurol* 2011;258:203–211, doi: http://dx.doi.org/10.1007/s00415-010-5708-z.

95. Garcia-Martin E, Martinez C, Alonso-Navarro H, et al. Gamma-aminobutyric acid GABRA4, GABRE, and GABRQ receptor polymorphisms and risk for essential tremor. *Pharmacogenet Genomics* 2011;21:436–439, doi: http://dx.doi.org/10.1097/FPC.0b013e328345bec0.

96. Ledesma MC, Garcia-Martin E, Alonso-Navarro H, et al. The nonsynonymous Thr105Ile polymorphism of the histamine N-methyltransferase is associated to the risk of developing essential tremor. *Neuromolecular Med* 2008;10:356–361, doi: http://dx.doi.org/10.1007/s12017-008-8040-3.

97. Keeling BH, Vilarino-Guell C, Soto-Ortolaza AI, et al. Histamine N-methyltransferase Thr105Ile is not associated with Parkinson's disease or essential tremor. *Parkinsonism Relat Disord* 2010;16:112–114, doi: http://dx.doi.org/10.1016/j.parkreldis.2009.08.011.

98. Zimprich A. Phenocopies in families with essential tremor and restless legs syndrome challenge Mendelian laws. Epigenetics might provide answers. *Parkinsonism Relat Disord* 2012.

99. Prakash KM, Tan EK. Validity of family history in essential tremor. *Parkinsonism Relat Disord* 2008;14:151–153, doi: http://dx.doi.org/10.1016/j.parkreldis.2007.05.007.

100. Nguyen HV, Ngian V, Cordato D, Shen Q, Chan DK. Quality of life in a random sample of community dwelling older patients with essential tremor. *Acta Neurol Scand* 2007;116:289–292, doi: http://dx.doi.org/10.1111/j.1600-0404.2007.00863.x.

101. Louis ED, Wendt KJ, Ford B. Senile tremor. What is the prevalence and severity of tremor in older adults? *Gerontology* 2000;46:12–16, doi: http://dx.doi.org/10.1159/000022127.

102. Benito-Leon J, Bermejo-Pareja F, Louis ED. Incidence of essential tremor in three elderly populations of central Spain. *Neurology* 2005;64:1721–1725, doi: http://dx.doi.org/10.1212/01.WNL.0000161852.70374.01.

103. Coriell Institute. http://ccr.coriell.org/Sections/Collections/NINDS/Population.aspx?PgId=194&coll=ND. 2012.

104. Schrag A, Munchau A, Bhatia KP, Quinn NP, Marsden CD. Essential tremor: an overdiagnosed condition? *J Neurol* 2000;247:955–959, doi: http://dx.doi.org/10.1007/s004150070053.

105. Jain S, Lo SE, Louis ED. Common misdiagnosis of a common neurological disorder: how are we misdiagnosing essential tremor? *Arch Neurol* 2006;63:1100–1104, doi: http://dx.doi.org/10.1001/archneur.63.8.1100.

106. Davis CH, Jr., Kunkle EC. Benign essential (heredofamilial) tremor. *AMA Arch Intern Med* 1951;87:808–816, doi: http://dx.doi.org/10.1001/archinte.1951.03810060035004.

107. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. *Neurology* 1967;17:427–442, doi: http://dx.doi.org/10.1212/WNL.17.5.427.

108. Tse W, Libow LS, Neufeld R, et al. Prevalence of movement disorders in an elderly nursing home population. *Arch Gerontol Geriatr* 2008;46:359–366, doi: http://dx.doi.org/10.1016/j.archger.2007.05.008.

109. Critchley EM. Essential tremor. *Lancet* 2007;369(9580):2157, doi: http://dx.doi.org/10.1016/S0140-6736(07)61004-6.

110. Elble RJ, Higgins C, Elble S. Electrophysiologic transition from physiologic tremor to essential tremor. *Mov Disord* 2005;20:1038–1042, doi: http://dx.doi.org/10.1002/mds.20487.

111. Cersosimo MG, Koller WC. Essential tremor. In: Watts RL and Koller WC (eds.) *Movement Disorders: Neurological Principles and Practice*, second edition. 2004: McGraw-Hill; pp. 431–458.

112. Testa CM, Rosen AR, Wichmann T, Levey AI, Bouzyk M, Factor SA. Essential tremor phenotyping and molecular genetics: database cases and a new large pedigree. *Mov Disord* 2006;21:S405.

113. Louis ED, Ottman R, Ford B, et al. The Washington Heights-Inwood Genetic Study of Essential Tremor: methodologic issues in essential-tremor research. *Neuroepidemiology* 1997;16:124–133, doi: http://dx.doi.org/10.1159/000109681.

114. MacMahon B, Pugh TF. Causes and entities of disease. In: Clark DW and MacMahon B (eds.) *Preventive Medicine*. 1967, Boston: Little, Brown; p 11–18. (Available in Evolution of Epidemiologic Ideas: Annotated Readings on Concepts and Methods, 1987).

115. Elble RJ. Report from a U.S. conference on essential tremor. *Mov Disord* 2006;21:2052–2061, doi: http://dx.doi.org/10.1002/mds.21157.

116. Jankovic J. Essential tremor: a heterogenous disorder. *Mov Disord* 2002;17:638–644, doi: http://dx.doi.org/10.1002/mds.10221.

117. Adler CH, Shill HA, Beach TG. Essential tremor and Parkinson's disease: lack of a link. *Mov Disord* 2011;26:372–377, doi: http://dx.doi.org/10.1002/mds.23509.

118. Fekete R, Jankovic J. Revisiting the relationship between essential tremor and Parkinson's disease. *Mov Disord* 2011;26:391–398, doi: http://dx.doi.org/10.1002/mds.23512.

119. Shahed J, Jankovic J. Exploring the relationship between essential tremor and Parkinson's disease. *Parkinsonism Relat Disord* 2007;13:67–76, doi: http://dx.doi.org/10.1016/j.parkreldis.2006.05.033.

120. Spanaki C, Plaitakis A. Essential tremor in Parkinson's disease kindreds from a population of similar genetic background. *Mov Disord* 2009;24:1662–1668, doi: http://dx.doi.org/10.1002/mds.22655.

121. Wider C, Ross OA, Wszolek ZK. Genetics of Parkinson disease and essential tremor. *Curr Opin Neurol* 2010;23:388–393.

122. Costello S, Bordelon Y, Bronstein J, Ritz B. Familial associations of Alzheimer disease and essential tremor with Parkinson disease. *Eur J Neurol* 2010;17:871–878, doi: http://dx.doi.org/10.1111/j.1468-1331.2010.02974.x.

123. Puschmann A, Pfeiffer RF, Stoessl AJ, et al. A family with Parkinsonism, essential tremor, restless legs syndrome, and depression. *Neurology* 2011;76:1623–1630, doi: http://dx.doi.org/10.1212/WNL.0b013e318219fb42.

124. Zhang J, Carr CW, Rigamonti D, Badr A. Genome-wide linkage scan maps ETINPH gene to chromosome 19q12-13.31. *Hum Hered* 2010;69:262–267, doi: http://dx.doi.org/10.1159/000288711.

125. Cohen O, Pullman S, Jurewicz E, Watner D, Louis ED. Rest tremor in patients with essential tremor: prevalence, clinical correlates, and electrophysiologic characteristics. *Arch Neurol* 2003;60:405–410, doi: http://dx.doi.org/10.1001/archneur.60.3.405.

126. Rajput AH, Rozdilsky B, Ang L, Rajput A. Significance of parkinsonian manifestations in essential tremor. *Can J Neurol Sci* 1993;20:114–117.

127. Koller WC, Rubino FA. Combined resting-postural tremors. *Arch Neurol* 1985;42:683–684, doi: http://dx.doi.org/10.1001/archneur.1985.04060070073019.

128. Bain P, Brin M, Deuschl G, et al. Criteria for the diagnosis of essential tremor. *Neurology* 2000;54(11 Suppl 4):S7.

129. Louis ED, Wendt KJ, Pullman SL, Ford B. Is essential tremor symmetric? Observational data from a community-based study of essential tremor. *Arch Neurol* 1998;55:1553–1559, doi: http://dx.doi.org/10.1001/archneur.55.12.1553.

130. Putzke JD, Whaley NR, Baba Y, Wszolek ZK, Uitti RJ. Essential tremor: predictors of disease progression in a clinical cohort. *J Neurol Neurosurg Psychiatry* 2006;77:1235–1237.

131. Hubble JP, Busenbark KL, Pahwa R, Lyons K, Koller WC. Clinical expression of essential tremor: effects of gender and age. *Mov Disord* 1997;12:969–972, doi: http://dx.doi.org/10.1002/mds.870120620.

132. Stolze H, Petersen G, Raethjen J, Wenzelburger R, Deuschl G. The gait disorder of advanced essential tremor. *Brain* 2001;124:2278–2286, doi: http://dx.doi.org/10.1093/brain/124.11.2278.

133. Klebe S, Stolze H, Grensing K, Volkmann J, Wenzelburger R, Deuschl G. Influence of alcohol on gait in patients with essential tremor. *Neurology* 2005;65:96–101, doi: http://dx.doi.org/10.1212/01.wnl.0000167550.97413.1f.

134. Singer C, Sanchez-Ramos J, Weiner WJ. Gait abnormality in essential tremor. *Mov Disord* 1994;9:193–196, doi: http://dx.doi.org/10.1002/mds.870090212.

135. Parisi SL, Heroux ME, Culham EG, Norman KE. Functional mobility and postural control in essential tremor. *Arch Phys Med Rehabil* 2006;87:1357–1364, doi: http://dx.doi.org/10.1016/j.apmr.2006.07.255.

136. Elble RJ, Dubinsky RM, Ala T. Alzheimer's disease and essential tremor finally meet. *Mov Disord* 2007;22:1525–1527, doi: http://dx.doi.org/10.1002/mds.21595.

137. Schiebler S, Schmidt A, Zittel S, et al. Arm tremor in cervical dystonia–is it a manifestation of dystonia or essential tremor? *Mov Disord* 2011;26:1789–1792, doi: http://dx.doi.org/10.1002/mds.23837.

138. Hedera P, Phibbs FT, Fang JY, Cooper MK, Charles PD, Davis TL. Clustering of dystonia in some pedigrees with autosomal dominant essential tremor suggests the existence of a distinct subtype of essential tremor. *BMC Neurol* 2010;10:66, doi: http://dx.doi.org/10.1186/1471-2377-10-66.

139. Louis ED, Borden S, Moskowitz CB. Essential tremor centralized brain repository: diagnostic validity and clinical characteristics of a highly selected group of essential tremor cases. *Mov Disord* 2005;20:1361–1365, doi: http://dx.doi.org/10.1002/mds.20583.

140. Frazer KA, Murray SS, Schork NJ, Topol EJ. Human genetic variation and its contribution to complex traits. *Nat Rev Genet* 2009;10:241–251, doi: http://dx.doi.org/10.1038/nrg2554.

141. Schork NJ, Murray SS, Frazer KA, Topol EJ. Common vs. rare allele hypotheses for complex diseases. *Curr Opin Genet Dev* 2009;19:212–219, doi: http://dx.doi.org/10.1016/j.gde.2009.04.010.

142. Li B, Leal SM. Discovery of rare variants via sequencing: implications for the design of complex trait association studies. *PLoS Genet* 2009;5:e1000481, doi: http://dx.doi.org/10.1371/journal.pgen.1000481.

143. Tan EK, Schapira AH. Hunting for genes in essential tremor. *Eur J Neurol* 2008;15:889–890, doi: http://dx.doi.org/10.1111/j.1468-1331.2008.02226.x.

144. Bain PG, Findley LJ, Thompson PD, et al. A study of hereditary essential tremor. *Brain* 1994;117:805–824, doi: http://dx.doi.org/10.1093/brain/117.4.805.

145. Ma S, Davis TL, Blair MA, et al. Familial essential tremor with apparent autosomal dominant inheritance: should we also consider other inheritance modes? *Mov Disord* 2006;21:1368–1374, doi: http://dx.doi.org/10.1002/mds.20950.

146. Jablonka E, Raz G. Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Q Rev Biol* 2009;84:131–176, doi: http://dx.doi.org/10.1086/598822.

147. Brink RA. A Genetic Change Associated with the R Locus in Maize Which Is Directed and Potentially Reversible. *Genetics* 1956;41:872–889.

148. Bennett ST, Wilson AJ, Esposito L, et al. Insulin VNTR allele-specific effect in type 1 diabetes depends on identity of untransmitted paternal allele. The IMDIAB Group. *Nat Genet* 1997;17:350–352, doi: http://dx.doi.org/10.1038/ng1197-350.

149. Lister R, Pelizzola M, Dowen RH, et al. Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* 2009;462:315–322, doi: http://dx.doi.org/10.1038/nature08514.

150. Ng SB, Turner EH, Robertson PD, et al. Targeted capture and massively parallel sequencing of 12 human exomes. *Nature* 2009;461:272–276, doi: http://dx.doi.org/10.1038/nature08250.

151. Kumar KR, Lohmann K, Klein C. Genetics of Parkinson disease and other movement disorders. *Curr Opin Neurol* 2012;25:466–474, doi: http://dx.doi.org/10.1097/WCO.0b013e3283547627.

152. McCulloch CC, Kay DM, Factor SA, et al. Exploring gene-environment interactions in Parkinson's disease. *Hum Genet* 2008;123:257–265, doi: http://dx.doi.org/10.1007/s00439-008-0466-z.

153. Tomiyasu A, Nakamura M, Ichiba M, et al. Novel pathogenic mutations and copy number variations in the VPS13A gene in patients with chorea-acanthocytosis. *Am J Med Genet B Neuropsychiatr Genet* 2011;156B:620–631.

154. Nuytemans K, Theuns J, Cruts M, Van Broeckhoven C. Genetic etiology of Parkinson disease associated with mutations in the SNCA, PARK2, PINK1, PARK7, and LRRK2 genes: a mutation update. *Hum Mutat* 2010;31:763–780, doi: http://dx.doi.org/10.1002/humu.21277.

155. Choy KW, Setlur SR, Lee C, Lau TK. The impact of human copy number variation on a new era of genetic testing. *Bjog* 2010;117:391–398, doi: http://dx.doi.org/10.1111/j.1471-0528.2009.02470.x.

156. Paulsen JS, Langbehn DR, Stout JC, et al. Detection of Huntington's disease decades before diagnosis: the Predict-HD study. *J Neurol Neurosurg Psychiatry* 2008;79:874–880, doi: http://dx.doi.org/10.1136/jnnp.2007.128728.

157. Factor SA, Steenland NK, Higgins DS, et al. Disease-related and genetic correlates of psychotic symptoms in Parkinson's disease. *Mov Disord* 2011;26:2190–2195, doi: http://dx.doi.org/10.1002/mds.23806.

158. Ross OA, Soto-Ortolaza AI, Heckman MG, et al. Association of LRRK2 exonic variants with susceptibility to Parkinson's disease: a case-control study. *Lancet Neurol* 2011;10:898–908, doi: http://dx.doi.org/10.1016/S1474-4422(11)70175-2.

159. Latourelle JC, Hendricks AE, Pankratz N, et al. Genomewide linkage study of modifiers of LRRK2-related Parkinson's disease. *Mov Disord* 2011;26:2039–2044, doi: http://dx.doi.org/10.1002/mds.23781.

160. Renegar G, Webster CJ, Stuerzebecher S, et al. Returning genetic research results to individuals: points-to-consider. *Bioethics* 2006;20:24–36, doi: http://dx.doi.org/10.1111/j.1467-8519.2006.00473.x.