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

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
study 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): 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%. 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. 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 ascertainment rate of 100%. Methodology is one factor. Only a fraction of ET cases are thus included.

Recombination during meiosis is unlikely. The farther apart alleles are on the same chromosome, and are close enough together that chromosomal markers) or loci (areas of DNA containing many genes) that are linked in two North American families with ET plus dystonia but not families with classic ET alone (OMIM 611456). Only ETM1 has been independently confirmed. 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<6.4×10^{-6}. 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. 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. A subsequent association study of three polymorphic markers within ETM2 in a Czech cohort was negative, as was a case–control study of microsatellite markers across ETM2 in a Latvian cohort. The only published ETM3 follow-up confirmation attempts are locus exclusions in some ET families. 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. 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. 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-richrepeat- 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 13,394 controls, then confirmed one SNP (rs9652490) in Icelandic, US, and European ET cohorts. 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, including European, North American, Latvian, and Asian cohorts. At least one study used only definite ET cases. 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. 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, Spanish, or French Canadian cohorts. A separate Chinese study found no significant association of rs9652490 with ET; however, a meta-analysis combining the initial data with published 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.

The overall results in LINGO1 could be due to population-specific differences, 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.

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 (rs1412292). In addition, two haplotype-tagged SNPs in LINGO2 (rs10812774, rs7033345) influenced ET age of onset. 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. Reported LINGO2 risk associations are significant but relatively weak.

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. While initial work pointed to a strong association between this polymorphism and at least some ET, 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. The HS1-BP3 828C>G variant was not associated with either ET or PD risk in our cohorts. 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; an independent study of Spanish subjects also observed an association with both ET risk and ET age of onset. 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, or linkage with DRD3 in ET families. 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 GB4, fragile X, and spinocerebellar ataxias, as well as a few unconfirmed positive reports [microtubule-associated protein tau MAPT for example]), 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 (REPI) in the gene encoding z-synuclein (SNCA) and ET as well as PD. SVCA mutations and genomic multiplications play a clear role in rare forms of inherited PD. REP1 allele length variants are associated with idiopathic PD in multiple studies. However, a subsequent independent study of SNCA and ET (Italian cohort) did not find an association between ET risk and SNCA haplotypes. 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 | Notes                                                                 |
|------------|------|-----------|------------------|----------------|----------------------------------------------------------------------|
| **ETM1**   |      |           |                  |                |                                                                     |
| HS1-BP3 828C→G variant | 49   | 92        | United States    | Negative       |                                                                     |
| Shatunov et al, 2005<sup>63</sup> |      |           |                  |                |                                                                     |
| HS1-BP3 828C→G variant | 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 |
| Higgins et al, 2006<sup>61</sup> |      |           |                  |                |                                                                     |
| HS1-BP3 828C→G variant | 222  | 132       | United States    | Negative       |                                                                     |
| Deng et al, 2005<sup>62</sup> |      |           |                  |                |                                                                     |
| **ETM2**   |      |           |                  |                |                                                                     |
| ETM2 sequence variants | 30   | 30        | Korean           | Positive       |                                                                     |
| Kim et al, 2005<sup>37</sup> |      |           |                  |                |                                                                     |
| ETM2 polymorphisms | 61   | 68        | Czech            | Negative       |                                                                     |
| Zahorakova et al, 2010<sup>38</sup> |      |           |                  |                |                                                                     |
| DRD3 312A→G variant | 104  | 116       | Latvian          | Negative       |                                                                     |
| Inashkina et al, 2008<sup>39</sup> |      |           |                  |                |                                                                     |
| DRD3 312A→G variant | 116  | 158       | Italian          | Negative       |                                                                     |
| Vitale et al, 2008<sup>40</sup> |      |           |                  |                |                                                                     |
| DRD3 312A→G variant | 163  | 192       | Singaporean      | Negative       |                                                                     |
| Tan et al, 2007<sup>41</sup> |      |           |                  |                |                                                                     |
| DRD3 312A→G variant | 201  | 282       | Spanish          | Positive       |                                                                     |
| Garcia-Martin et al, 2009<sup>42</sup> |      |           |                  |                |                                                                     |
| DRD3 312A→G variant | 276  | 184       | United States    | Positive       |                                                                     |
| Jeanneteau et al, 2006<sup>43</sup> | 30   | 50        | French           |                |                                                                     |
| DRD3 312A→G variant | 299  | 528       | German, Danish,  | Negative       |                                                                     |
| Lorenz et al, 2009<sup>44</sup> |      |           | French           |                |                                                                     |
| DRD3 312A→G variant | 433  | 272       | United States    | Negative       |                                                                     |
| Blair et al, 2008<sup>45</sup> |      |           |                  |                |                                                                     |
| Study | ET n | Control n | ET Cohort Source | Overall Result |
|-------|------|-----------|------------------|---------------|
| LINGO genes |      |           |                  |               |
| *LINGO1* rs9652490 and rs11856808 | 109  | 430       | Chinese Han      | Negative      |
| Zuo et al, 2010<sup>54</sup> |      |           |                  |               |
| *LINGO1* rs9652490 | 117  | 160       | Chinese          | Negative      |
| Wu et al, 2011<sup>57</sup> | Note combining with Tan et al<sup>53</sup> plus unpublished data into 307 ET and 804 control, meta-analysis positive |
| *LINGO1* rs9652490 | 141  | 130       | Latvian          | Positive      |
| Radovica et al, 2012<sup>52</sup> | 9 other SNPs including rs11856808 negative |
| *LINGO4* variants | 150  | 300       | Chinese Han      | Negative      |
| Liang et al, 2012<sup>59</sup> |      |           |                  |               |
| *LINGO1* rs9652490 | 190  | 733       | Singaporean      | Positive      |
| Tan et al, 2009<sup>53</sup> |      |           |                  |               |
| *LINGO1* rs9652490 and rs11856808 | 226  | 1117      | Spanish          | Negative      |
| Lorenzo-Betancor et al, 2011<sup>55</sup> |      |           |                  |               |
| *LINGO1* SNPs | 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<sup>46,50,51,53</sup> also positive |
| Clark et al, 2010<sup>51</sup> |      |           |                  |               |
| *LINGO1* rs9652490 and rs11856808 | 259  | 479       | French Canadian  | Negative      |
| Bourassa et al, 2011<sup>56</sup> |      |           |                  |               |
| *LINGO2* variants | 327  | 499       | Chinese, Singaporean Positive for rs7033345 and rs10812774 of 8 variants | |
| Wu et al, 2011<sup>58</sup> |      |           |                  |               |
| *LINGO1* SNPs | 332  | 574       | German, French   | Positive for rs9652490 and 1 other Negative for rs11856808 and others out of 10 SNPs total |
| Thier et al, 2010<sup>68</sup> |      |           |                  |               |
| *LINGO1* rs9652490 | 356  | 428       | North American   | Positive      |
| Vilarino-Guell et al, 2010<sup>50</sup> |      |           |                  |               |
REP1 lengths: the four dinucleotide repeats constituting REP1 all varied; thus, alleles with identical lengths may represent varied dinucleotide sequences instead of homozygous alleles. 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.

| Study | ET n | Control n | ET Cohort Source | Overall Result |
|-------|------|-----------|------------------|----------------|
| GWAS  | 452  | 14,394    | Icelandic        | Positive for LINGO1 rs9652490 and rs11856808 in initial cohort; rs9652490 only in confirmatory cohorts |
| Stefansson et al, 2009⁴⁶ | 281  | 1188      | Austrian, German, United States |  |
| LINGO1 and LINGO2 variants | 1247 | 642       | North American   | Positive for LINGO1 rs9652490 and 4 others of 16 total, and LINGO2 rs1412229 alone of 21. |
| Vilarino-Guell et al, 2010⁹⁹ |  |

| SNCA |
|------|
| REP1 length | 46  | 100 | United States | Positive |
| Tan et al, 2000⁷⁵ |  |
| SNCA variants | 106 | 90  | Italian       | Negative |
| Pigullo et al, 2003⁹⁰ |  |
| SNCA variants | 647 | 1285 | North American | Negative |
| Ross et al, 2011⁹¹ |  |

| Other variants |
|----------------|
| GABAAR subunit genes variants | 200 | 250 | Spanish | Negative |
| Garcia-Martin et al, 2011⁹⁴⁹⁵ |  |
| GABAAR subunits and GABA transporter genes variants | 503 | 818 | German, Dutch | Negative |
| Thier et al, 2011⁹³ |  |
| HNMT rs11558538 | 204 | 295 | Spanish | Positive |
| Ledesma et al, 2008⁹⁶ |  |
| HNMT rs11558538 | 338 | 409 | North American | Negative |
| Keeling et al, 2010⁹⁷ |  |

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 is 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.
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. 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. Variants in GABAAR subtype genes were also not associated with ET risk studies in Spanish samples.

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

**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. 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, and tremor self-reporting is often inaccurate; 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. 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. 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. The most common alternative diagnoses are 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. 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.
**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    | Creates false positives | Test different phenotype features or subsets against genotype data (see also Table 3). | How to make phenotype groups more genetically homogeneous is currently unknown. | 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. |
| Multiple genetic loci | Lowers LOD score | Expanding or narrowing possible phenotypes may or may not increase genetic homogeneity within groups. | Experimental design goals are to broaden testable hypotheses, thus move past the potential stalemate of as yet unknown phenotype-genotype connections. |
| Unknown genotypes  | Increased type 1 error | Use inclusive varied phenotype as outcome rather than narrow ET definition. | | |
|                    | One genetic variant may have large phenotypic range (genocopies). | | | |
| **Increasing prevalence with age** | Creates false negatives | 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. |
| | Lowers LOD score | | | |
| | Increased type 2 error | | | |
| **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 | 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 | Both subject and researcher resources impact feasibility of extended direct exams. | |
| **ET misdiagnoses:** | False positives and false negatives | Larger sample sizes to improve ability to detect associations Utilizing possible / probable / definite categories, restricting analysis to definite cases | 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. |
| Other disorders; mild tremor versus normal | | 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. | | |

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.\textsuperscript{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.\textsuperscript{112}

One approach is to utilize a possible/probable/definite scheme for ET research diagnosis,\textsuperscript{18,113} as in the twin study above.\textsuperscript{16} At least three of the association studies above restricted ET cases to TRIG criteria definite\textsuperscript{40,90} or probable and definite ET.\textsuperscript{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.\textsuperscript{51,91}

The Shatunov et al\textsuperscript{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\textsuperscript{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.”\textsuperscript{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.\textsuperscript{10,115,116} 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.\textsuperscript{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\textsuperscript{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.\textsuperscript{10,115,116} Early attempts to clearly separate ET and PD also acknowledge “exceptional cases” that could represent an ET–parkinsonism overlap.\textsuperscript{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” Early age of onset | May not be currently useful, often ill defined. Retrospective data, large differences in actual tremor age of onset versus bothersome increased tremor symptoms reported as age of onset. | If “familial” subset used, clear definition with how family history data obtained. Longitudinal prospective data would strengthen this considerably. |
| Classic ET versus complicated ET ET-PD ET-parkinsonism ET-dystonia | ET is tremor in isolation by clinical definition ET and PD may or may not be related disorders; ET and PD are common mutual misdiagnoses. ET is tremor in isolation by clinical definition; ET and PD may or may not be related disorders 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. | | Use of research phenotype features rather than clinical diagnosis criteria. Record ET, PD, and dystonia exam features; then able to exclude ET-PD or dystonia cases from genetics studies, or focus on an ET-PD, ET-parkinsonism, or ET-dystonia subset depending on hypothesis. 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. Epigenetic or other novel hypotheses for disease transmission | Detecting different types of genetic risk factors and causal mutations Addressing non-mendelian inheritance at a complex biological data level | Collaborative phenotype-genotype studies with multiple research groups to achieve large sample numbers and rich prospective longitudinal phenotype data. 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. Return genetic research results information to research subjects and ET community. | ET community concerns may not be relevant to phenotype-genotype experiment design. How to return complex genetic data information to research subjects is not straightforward. | Understand ET community research goals, ET subject observations about ET phenotype and inheritance. 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 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 al91 for one example) but far from standard in ET genetics work.91 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.15,48,91,118–122 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.12,125–127 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.10,115,128 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.1,18,23,25 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.10,115,128 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 specified 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 al137 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.140 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. 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 al36 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;121 pathophysiology could therefore involve rare genetic variants in combination, instead of a few common variants. 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,10 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,15,143 although complex multigenetic modes of inheritance cannot yet be excluded.22,36,43,116,145 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. 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. As more and more familial linkage studies
returned negative results, some suspected “too many” affecteds for a straightforward autosomal dominant mechanism. 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.

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. This type of inheritance can occur in humans, through unclear mechanisms (reviewed in Zimprich). 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. 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. Long described in plants, the role of paramutation in mammals, particularly humans, is postulated from observations in diabetes mellitus but is uncertain. Zimprich puts forward a framework of primarily epigenetic and paramutation inheritance, rather than genetic state, accounting for ET pedigree phenotype patterns. 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.

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. Uncovering “missing heritability” in ET may require researchers to pursue rare rather than common causal genetic variants, and/or many genetic risk factors. 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 causative mutation. High-throughput sequencing can be restricted to exons (exome sequencing). 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.

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. Already, meta-analyses leveraging multiple independent GWAS datasets in PD have set an example for detecting genetic risk factors with small effect sizes. Considering genetic and environmental risk factor data together is another potentially fruitful approach 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. 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. New array technologies are greatly expanding the ability to detect copy number variants. 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.

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, 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; considering intronic and regulatory as coding regions; utilizing a variety of linkage and association analysis techniques. To some extent, genetics efforts are also moving on from older phenotype strategies: for example, using definite ET for affected status and including data on dystonia. 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 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; the GWAS required input and multiple confirmatory cohorts from collaborators.36 Of the association studies reviewed in Table 1, the only ones above an ET n of 5001 or 100019 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 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 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, and PD when advocating for the level of ET phenotype–genotype work necessary to create scientific advances in the field.

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A comprehensive review of the genetic research challenges associated with essential tremor and Parkinson's disease reveals several key findings. Essential tremor (ET) and Parkinson's disease (PD) share some genetic similarities, particularly in their susceptibility to mutations in genes encoding proteins involved in nigrostriatal dopamine neurotransmission. Studies have identified variants in genes such as Dopamine Receptor D3 (DRD3), Lingo1 (LINGO1), and Lingo2 (LINGO2) that are associated with susceptibility to both conditions. For instance, a LINGO1 polymorphism is associated with ET in the North American population, as reported in *Parkinsonism Relat Disord* 2010;16:109–111, doi: 10.1016/j.parkreldis.2009.08.006. Similarly, the DRD3 variant is associated with PD risk, as indicated in *Neurology* 2005;65:915–919, doi: 10.1212/01.WNL.0000173033.32535.23. These findings underscore the genetic overlap between ET and PD, suggesting that genetic research in ET may provide insights into PD susceptibility and vice versa.

Furthermore, the identification of genetic variants associated with ET has implications for understanding the pathophysiology of PD. For example, the LINGO1 polymorphism, which has been associated with ET, is also linked to PD risk. This finding suggests a potential role for LINGO1 in the development of PD, and further research is needed to elucidate the specific mechanisms by which these variants contribute to disease susceptibility. In addition, the discovery of genetic variants associated with ET and PD highlights the importance of genetic studies in understanding the molecular basis of these disorders, which can potentially lead to the development of targeted therapeutic strategies.

Overall, the genetic research challenges in ET and PD are complex and multifaceted, requiring collaboration between geneticists, clinicians, and basic scientists working in these fields. As genetic technologies advance, the ability to identify and understand the genetic factors associated with these disorders will likely improve, leading to a better understanding of their pathogenesis and ultimately to improved therapeutic options for patients.
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