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Generalized, Focal, and Combined Epilepsies in Families: New Evidence for Distinct Genetic Factors

Running head: Generalized, Focal, and Combined Epilepsies in Families

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ABSTRACT

Objective: To determine the roles of shared and distinct genetic influences on generalized and focal epilepsy operating in individuals who manifest features of both types (*combined epilepsies*) and in families manifesting both generalized and focal epilepsies in separate individuals (*mixed families*).

Methods: We analyzed the deeply-phenotyped Epi4K cohort of multiplex families (≥3 affected individuals per family) using methods that quantify the aggregation of phenotypes within families and the relatedness of individuals with different phenotypes within family pedigrees.

Results: The cohort included 281 families containing 1,021 individuals with generalized (n = 484), focal (304), combined (51), or unclassified (182) epilepsies. The odds of combined epilepsy was higher in relatives of participants with combined epilepsy than in relatives of those with other epilepsy types (OR 5.2, 95%CI 1.7, 16.1, p = 0.004). Individuals with combined epilepsy co-occurred in families more often than expected by chance (p = 0.03). Within mixed families,

individuals with each type of epilepsy were more closely related to relatives with the same type than to relatives with other types ($p < 0.001$).

Significance: These findings suggest that distinct genetic influences underlie the recently-recognized entity of combined epilepsies, just as generalized epilepsies and focal epilepsies each have distinct genetic influences. Mixed families may in part reflect chance co-occurrence of these distinct genetic influences. These conclusions have important implications for molecular genetic studies aimed at identifying genetic determinants of the epilepsies.

KEY POINTS

- Combined generalized and focal epilepsy in a single individual, recently recognized as a distinct epilepsy type in the 2017 ILAE Classification, tends to aggregate in families, suggesting unique genetic determinants distinct from other types of epilepsy.
- Combined epilepsy in families was not more closely associated with generalized epilepsies than with focal epilepsies.
- Combined epilepsy was not more likely to occur in mixed families than in otherwise homogeneous families.
- Mixed families that contain generalized epilepsy and focal epilepsy in separate individuals need not imply shared genetic determinants, and may reflect chance co-occurrence of distinct genetic determinants for different epilepsy phenotypes.

INTRODUCTION

The division of epilepsies into two main types, focal and generalized, is a basic tenet of epileptology. This dichotomy determines the clinical approach to investigation and treatment, and has been enshrined in the classification of epilepsies for 50 years.[1,2] There are, however, patients with clear evidence of both focal-onset and generalized-onset seizures. These patients are now recognized in the 2017 ILAE Classification as having a *combined* focal and generalized epilepsy type.[3] Such cases fall into two broad groups; first, infants or children with epileptic encephalopathies (e.g., Dravet and Lennox-Gastaut syndromes) and second, older children or adults with both generalized and focal onset seizure types. Here we explore the genetics of the latter group.

Family studies suggest that focal and generalized epilepsies have both distinct and shared genetic determinants.[4–7] Monozygotic twins are most often concordant for epilepsy type, suggesting that each type has distinct genetic determinants, but discordant twins do occur.[4,5] Family studies have generally found greater increases in risk for the same versus different types of epilepsy in the relatives of probands with a given epilepsy type,[6,8,9] providing evidence for distinct genetic influences. Similarly, one study found that families containing multiple individuals with epilepsy were concordant for epilepsy type more often than expected by chance, providing further evidence for distinct genetic effects.[7] However, findings have been somewhat inconsistent,[6,10] and mixed families containing relatives with both epilepsy types are not uncommon.[11] The genetic mechanisms that underlie individuals or families with combined generalized and focal epilepsy types are not well understood.

In this study, we sought to test a series of hypotheses about shared versus distinct genetic influences on generalized and focal epilepsies using methods that quantify the aggregation of phenotypes within family pedigrees.[12] We analyzed the well-characterized Epi4K cohort of multiplex families,[11] and evaluated both *individuals* with the combined epilepsy type and *families* containing separate participants with generalized or focal epilepsy (designated as *mixed families*).

For individuals with combined epilepsy, we considered three hypotheses (**Figure 1**). First, combined epilepsy may represent a subtype of generalized epilepsy with the same genetic determinants, as has been hypothesized for idiopathic photosensitive occipital epilepsy.[13] (Conversely, it could be a subtype of focal epilepsy, not shown in the figure.) Second, combined epilepsies may represent a blended or intermediate phenotype caused by the co-occurrence of distinct genetic determinants of generalized epilepsy and focal epilepsy within the same individual. Third, combined epilepsies may represent an independent phenotype, distinct from both focal and generalized epilepsies, with (at least in part) its own distinct genetic determinants.

For mixed families in which separate individuals manifest discordant epilepsy phenotypes, we considered two hypotheses (**Figure 2**). Individuals in mixed families may have *shared* genetic determinants for epilepsy of any type, with individual phenotypes determined by nongenetic factors. Alternatively, *distinct* genetic determinants of focal and generalized epilepsy may co-occur in these families, with individual phenotypes determined by which risk alleles an individual happens to inherit.

METHODS

Data Collection

Ascertainment and data collection of the Epi4K multiplex families have been described in detail elsewhere.[11] Families contained three or more relatives with epilepsy of unknown cause. Individuals with known acquired causes, structural brain lesions, and moderate or severe intellectual disability were excluded. Ascertainment occurred at multiple centers in the USA, Canada, Israel, Wales, Ireland, Australia and New Zealand. Individuals were classified by expert clinicians into epilepsy types based on review of all available clinical data (including seizure semiology, EEG and neuroimaging). These classifications were made without knowledge of other family members' epilepsy types. The following epilepsy types were included in this analysis: non-acquired focal epilepsy, genetic generalized epilepsy (GGE), combined focal and generalized epilepsy, and unknown epilepsy type. Combined epilepsy was diagnosed only when there was clear clinical and/or EEG evidence of both generalized and focal epilepsy in the individual. Non-specific semiologies, such as staring spells and tonic-clonic seizures, were not classified as generalized or focal unless supported by EEG data or more specific clinical features. Families were classified as Generalized if they contained only individuals with generalized (or unclassified) epilepsy; as Focal if they contained only individuals with focal (or unclassified) epilepsy; and as Mixed if they contained generalized and focal epilepsy in separate individuals, or if they contained any individuals with combined epilepsy. The original Epi4K cohort included an additional 22 families with genetic epilepsy with febrile seizures plus (GEFS+) that were not included in this analysis due to small sample size and because our primary interest here was exploring the genetic determinants of focal, generalized and combined epilepsies.

The Epi4K study was approved by the research ethics committee at each participating site and all participants provided informed consent to participate. The current analysis used deidentified data from that study and did not require additional ethics approval.

Statistical Analysis

We first tested our hypotheses regarding individuals with combined epilepsy (see Figure 1). To test for the aggregation of combined epilepsy within families, we estimated the odds ratio of combined epilepsy in relatives of participants with combined epilepsy compared to relatives of participants with other epilepsy types. Because these families were ascertained on the basis of

multiple affected individuals, none of those affected could be considered a "proband." Hence, we used the approach described previously of considering all possible pairs of affected relatives within each family.[14] Each affected relative was coded as having "combined epilepsy" or "other epilepsy." For each pair, one relative was randomly designated the independent (exposure) variable and the other designated the dependent (outcome) variable. We estimated the odds ratio using a logistic generalized estimating equation (GEE) model to account for the nonindependence of pairs within families.[15] Sensitivity analysis confirmed that this model was robust to the random designation of each relative in a pair as the dependent or independent variable, tested across 1,000 iterations.

As a separate test of aggregation of combined epilepsy within families we used permutation analyses, modified from the concordance method of Winawer et al.[16] Permutations were used to shuffle the phenotypes of affected individuals repeatedly and randomly; familial aggregation was assessed for each iteration, thus constructing a null distribution of the familial aggregation of phenotypes expected by chance based on the frequency of each phenotype in the dataset and the number of affected individuals in each family. These null distributions were then used to test whether an observed pattern of phenotypes between or within families was significantly different than expected by chance, using exact p-values.[12] First, we compared the observed co-occurrence of two or more individuals with combined epilepsy within the same family to the null distribution derived from 10,000 permutations of all phenotypes across all 281 families. Next, within the seven families containing two or more individuals with combined epilepsy, we compared the observed proportion of these individuals that were first-degree relatives to the null distribution derived from 10,000 permutations within these seven families.

We next assessed whether individuals with combined epilepsy were more likely to occur in families that otherwise manifested generalized epilepsies, focal epilepsies, or both. First, considering only individuals with generalized epilepsy or focal epilepsy, we classified each family as generalized, focal, or both (generalized epilepsy and focal epilepsy in separate individuals). Then we classified each family as either containing or not containing one or more individuals with combined epilepsy. We applied a chi-square test to the resulting 3x2 table, testing for enrichment of combined epilepsy in families that otherwise contained only generalized epilepsy, only focal epilepsy, or both.

Finally, we tested our hypotheses regarding mixed families that contain generalized epilepsy and focal epilepsy in separate individuals (see Figure 2). For this analysis we again considered all pairs of affected relatives within families as described above. For each pair we measured the relational distance between the two individuals (first degree = 1, second degree = 2, etc.). Statistical analyses were performed using binomial GEE models that treated pair type as outcome variable, relational distance as exposure variable, and family as clustering variable. Hypothesis testing was performed on each model using the Wald test.

Analyses were performed in the R programming language, using packages *Kinship2* for computing relatedness, *permute* for permutation analysis, and *geepack* for GEE models.

RESULTS

The characteristics of the Epi4K families cohort have been described in detail previously.[11] The numbers of families and individuals included in this analysis are shown in **Table 1**.

We first tested our hypotheses regarding individuals with combined epilepsy (see Figure 1). We interpreted aggregation of a phenotype within families as evidence of distinct genetic determinants specific to that phenotype (Figure 1C). The 51 individuals with combined epilepsy contributed to a total of 122 relative pairs. After randomly designating the exposure and outcome individual within each pair (see Methods), 54 pairs had only the exposure subject with combined epilepsy, 56 pairs had only the outcome subject with combined epilepsy, 12 pairs had both subjects with combined epilepsy, and 1,313 pairs had neither subject with combined epilepsy. The odds of combined epilepsy was higher in relatives of individuals with combined epilepsy (12/66 pairs, 18%) than in relatives of individuals with other types of epilepsy (56/1369 pairs, 4%; GEE-derived OR = 5.2, 95% CI 1.7, 16.1, p = 0.004).

The 51 individuals with combined epilepsy occurred in 42 families. Seven families contained two or more individuals with combined epilepsy (with one family containing four such individuals). This was more families than expected by chance (permutation p = 0.03) given the frequency of combined epilepsy in the cohort. Within these seven families, the affected individuals formed 12 unique pairs of relatives with combined epilepsy, which were first-degree relatives in 11/12 pairs (92%). This was not a higher proportion of first-degree pairs than expected by chance given the pedigree structures of these seven families (permutation p = 0.14).

To test the hypotheses that combined epilepsy is a subtype of generalized (or focal) epilepsy (Figure 1A) or represents the co-occurrence of genetic determinants of generalized and focal epilepsy in the same individual (Figure 1B), we assessed whether individuals with combined epilepsy were more likely to occur in families that manifest generalized epilepsies, focal epilepsies, or both. Of the 42 families that contained one or more individual with combined epilepsy, one family was entirely concordant for combined epilepsy in all four affected individuals. In the other 41 families, individuals with combined epilepsy had relatives with other phenotypes. Individuals with combined epilepsy were present in 18/136 families (13%) that otherwise contained only generalized epilepsy; in 17/80 families (21%) that otherwise contained only focal epilepsy; and in 6/64 families (9%) that contained both generalized and focal epilepsies in separate individuals. These proportions did not represent a significant enrichment of individuals with combined epilepsy in any of these family categories (chi-squared = 4.4, df = 2, p = 0.11).

Finally, we tested our hypotheses regarding mixed families that contain generalized epilepsy and focal epilepsy in separate individuals (see Figure 2). Within these 102 families, pairs of relatives with concordant phenotypes were more closely related than pairs with discordant phenotypes (Wald test = 11.0, df = 1, $p < 0.001$; **Figure 3**). This was driven largely by discordant pairs in which one relative had focal epilepsy and the other had generalized epilepsy, which were more distantly related than concordant pairs of either phenotype (Wald test = 12.5, df = 1, $p < 0.001$).

DISCUSSION

In this study of familial epilepsies, we found that the recently-recognized phenotype of combined epilepsy tends to aggregate in families, suggesting genetic influences that are distinct from other epilepsy types. We also found that in mixed families containing different epilepsy types in separate individuals, concordant phenotypes were more closely related than discordant phenotypes, suggesting distinct genetic influences on these epilepsy types even within mixed families. We did not find evidence that combined epilepsies are more closely associated with generalized epilepsies than with focal epilepsies, nor that combined epilepsies are more likely to occur in mixed families than in otherwise homogeneous families.

Combined generalized and focal epilepsy within individuals

Combined epilepsies have only recently been recognized by the ILAE classification of the epilepsies, and their genetic architecture is not known. It has been suggested that combined epilepsies may be subtypes of one of the other major epilepsy subtypes—for example, that idiopathic photosensitive occipital epilepsy (IPOE) is a form of generalized epilepsy with shared underlying genetic determinants, distinct from the determinants of focal epilepsy (Figure 1A).[13] We did not find evidence to support this hypothesis: combined epilepsies were equally likely to occur in families with generalized as with focal epilepsies, and did not aggregate more closely with one phenotype or the other within families.

A second hypothesis is that combined epilepsy occurs when separate risk alleles for generalized and focal epilepsy co-occur in a single individual (Figure 1B). However, if this were true, individuals with combined epilepsy should most often occur in families that also manifest generalized epilepsy and focal epilepsy in separate individuals, suggesting the presence of both sets of genetic influences within the family. We did not find evidence to support this hypothesis. In fact, although not statistically significant, the trend was toward families that contain generalzed and focal epilepsies in separate individuals being *less* likely to include individuals with combined epilepsy (9%) compared to generalized (13%) and focal (21%) families.

A third hypothesis is that combined epilepsies are an independent entity with genetic determinants distinct from those for generalized epilepsy or focal epilepsy (Figure 1C). In support of this hypothesis, we found that the odds of combined epilepsy was higher in relatives of participants with combined epilepsy than in relatives of those with other epilepsy types (OR 5.2, 95% CI 1.7, 16.1, p = 0.004), and that individuals with combined epilepsy co-occurred in families more often than expected by chance (p = 0.03). When they co-occurred in families, individuals with combined epilepsy were first degree relatives more often (11/12 pairs, 92%) than any other pair of phenotypes; our sample size was underpowered to explore this hypothesis. Future studies should investigate this question in sufficiently large cohorts with combined epilepsies.

We interpret these data as supporting the hypothesis that the combined epilepsy type has unique genetic influences independent of the genetic factors that influence generalized and focal epilepsies. The nature of these genetic determinants remains largely unknown. Recently, variants in the gene *RORB* were identified in individuals and families manifesting both photosensitive generalized seizures and focal (occipital) seizures.[17] Future studies should continue to explore the genetic architecture of the combined epilepsies.

Mixed Families

Although it is well established that generalized and focal epilepsies have distinct genetic determinants, mixed families have previously been interpreted as evidence of shared genetic mechanisms underlying different epilepsy phenotypes.[7,10,12] In a previous study of the Epi4K multiplex families, we noted that 21/102 (21%) of mixed families had discordant relatives separated by three or more meiotic events, and speculated that the discordant phenotypes in these families may be incidental, implying distinct genetic determinants.[11] Here we extended that finding with quantitative analyses of all relative pairs within these families and comparison of discordant pairs to concordant pairs. Our findings suggest that distinct genetic determinants may underlie different epilepsy phenotypes even when they occur within the same family. This may be due at least in part to the co-occurrence of distinct risk alleles for generalized epilepsy and focal epilepsy within a family, with individual phenotypes determined perhaps by the chance inheritance of these distinct risk alleles. Shared and distinct mechanisms are not mutually exclusive, and shared genetic determinants likely do play an important role in epilepsy, the clearest example being monozygotic twins with discordant phenotypes.[4,5] However, our findings highlight the role of distinct genetic determinants even within families, which has not previously been recognized as family studies tend to focus on shared genetics within families. This has important implications for molecular genetic studies aimed at identifying those genetic determinants.

Molecular genetic studies have begun to provide evidence for genes with both shared and distinct influences on different epilepsy phenotypes.[18–20] Some epilepsy-associated genes reliably cause a specific focal phenotype (eg, *LGI1* and lateral temporal lobe epilepsy, *CHRNA4* and sleep-related hypermotor epilepsy),[21,22] while others typically produce a generalized phenotype but may also raise risk for focal epilepsies (e.g., *SCN1A*, *SLC2A1*/GLUT1 deficiency).[23–25] Rare copy number variants raise risk of both focal and generalized epilepsies.[26]

Limitations

This study had several limitations. Our sample size of individuals with combined epilepsy was small and some analyses were likely underpowered. For this reason, we treated combined epilepsy as a single phenotypic entity, and were not able to study heterogeneity that likely exists among these individuals. We also lacked statistical power to evaluate specific syndromes within focal or

generalized epilepsies (e.g. childhood absence epilepsy; juvenile myoclonic epilepsy), which themselves tend to aggregate in families and may have syndrome-specific genetic determinants.[11,27] Next, we cannot exclude that discordant phenotypes within families are caused by differing environmental exposures rather than distinct genetic determinants. For example, distantly related individuals may live in different places or different times (across generations) with more disparate environmental exposures than first-degree relatives who share a household. However, there are no known environmental exposures that specifically determine focal versus generalized epilepsy types, and environmental effects are unlikely to fully explain our results. Although these subjects were carefully phenotyped by expert clinicians, some misclassification is possible; this might add noise to our data and make significant results more difficult to detect, but we do not believe it would bias our data toward spurious associations. Finally, our cohort consisted entirely of familial epilepsies and the extent to which these findings can be extended to sporadic epilepsies is uncertain.

Conclusions

In summary, our findings support the hypothesis that the combined generalized and focal epilepsy type has unique genetic determinants distinct from other epilepsy types, and that distinct genetic determinants of focal and generalized epilepsy phenotypes operate within mixed families, largely as 'chance' phenomena. Future molecular genetic studies should continue to explore the nature of shared and distinct genetic determinants of different epilepsy phenotypes in families and individuals.

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Potential Conflicts of Interest

None of the authors has any conflict of interest to disclose.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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FIGURE LEGENDS

Figure 1. Hypotheses regarding individuals with combined epilepsy.

(A) Combined focal and generalized epilepsy may be a subtype of generalized epilepsy, sharing the same genetic determinants and thus segregating together within families; (B) the combined phenotype may represent an intermediate phenotype resulting from combined genetic risks for both focal and generalized epilepsies within a given individual, in which case the combined phenotype should be most likely to occur in families that also contain generalized and focal epilepsy in separate in individuals; or (C) individuals with combined epilepsy may be a distinct phenotype, with genetic determinants distinct from those for focal or generalized epilepsies, in which case they will segregate with each other within families, distinct from other phenotypes.

Figure 2. Hypotheses regarding mixed families.

Solid colors represent diagnosed phenotypes and stippled colors represent theoretical underlying genetic determinants. The well-known familial aggregation of focal (A) and generalized (B) epilepsies within families suggests distinct genetic determinants for these epilepsy types. Within mixed families, two broad mechanisms are possible (and not mutually exclusive): (C) Mixed families are explained by co-occurrence of *distinct* genetic determinants for both focal and generalized epilepsies; (D) Alternatively, mixed families are explained by *shared* genetic determinants for epilepsy of all types, with individual phenotypes determined by other factors. If distinct genetic determinants for focal and generalized epilepsy operate within mixed families,

then concordant relatives will tend to be more closely related than discordant relatives. If not, then relational distance will not be associated with phenotypic concordance.
Abbreviations: Gen. = generalized epilepsy.

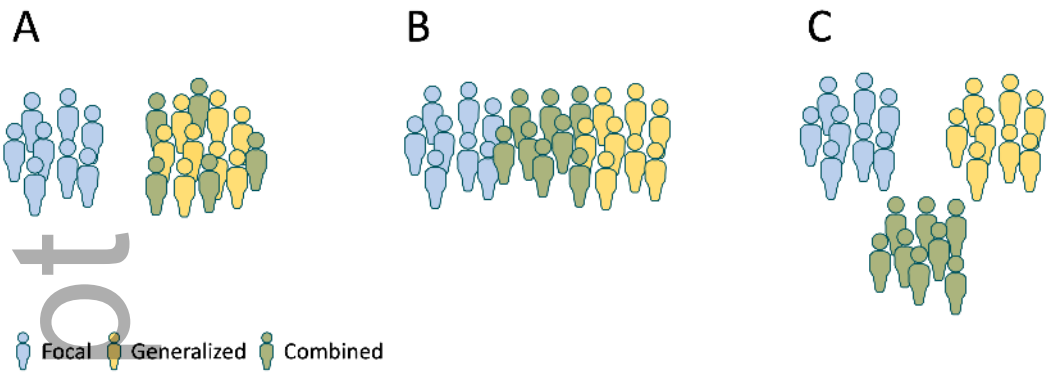
Figure 3. Relatedness of affected individuals in mixed families

Pairs of affected individuals with concordant phenotypes were more closely related than pairs with discordant phenotypes ($p < 0.001$).
Abbreviations: Comb. = combined epilepsy; Gen. = generalized epilepsy.

Table 1. Study Cohort.

Individual epilepsy types	**All families (N = 281)**	**Mixed families subset[a] (N = 102)**
Generalized	484	146
Focal	304	122
Combined	51	51
Unclassified	182	65
Total individuals	**1,021**	**384**

[a]Mixed families were defined as families that contained two or more different classifiable phenotypes in separate individuals, or contained any individuals with combined epilepsy.



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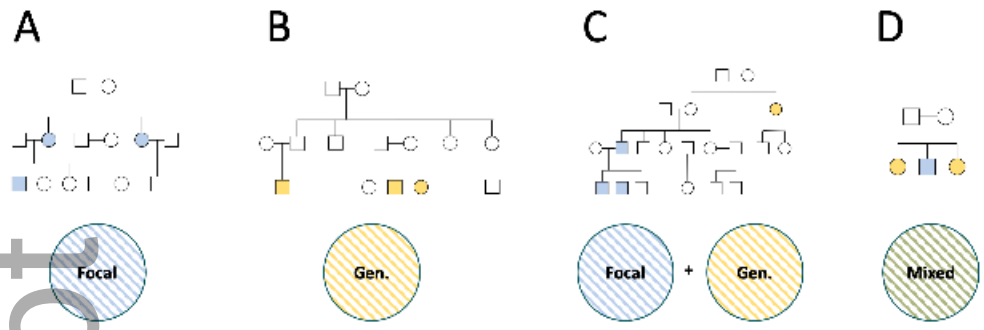

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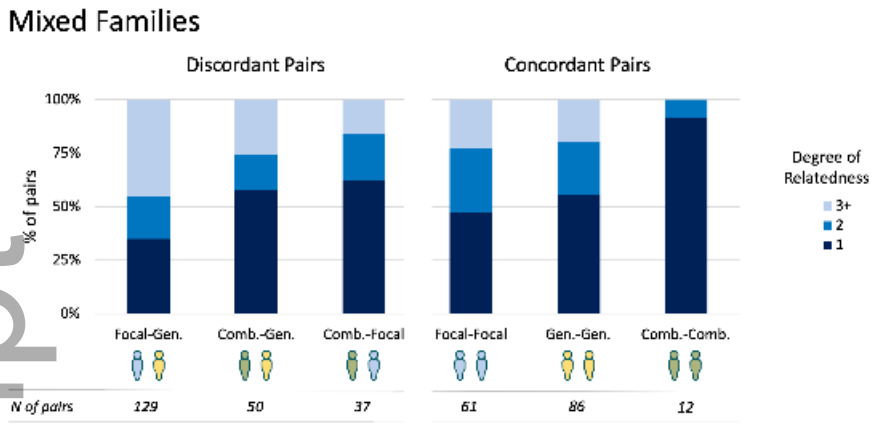

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