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Haplotype Distribution of the Human Phenylalanine Hydroxylase Locus in Scotland and Switzerland

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Summary

RFLP haplotypes at the phenylalanine hydroxylase (PAH) locus were determined in 45 nuclear Caucasian families from Switzerland and Scotland. The RFLPs at the PAH locus are highly informative, and prenatal diagnosis is possible in 85% of the families studied. The data were combined with the profiles previously observed in the Danish population, in order to study the variation in RFLP haplotype distribution among European populations. A total of 22 different haplotypes were observed in Denmark, Switzerland, and Scotland. Fifteen and 19 haplotypes are associated with the normal (non-PKU) and with the mutant chromosomes, respectively. The haplotype distribution and the allele frequency of normal chromosomes remain constant between Denmark, Switzerland, and Scotland. However, both the haplotype distribution and allele frequencies of mutant chromosomes show significant variation between the three countries. Our results suggest there may be additional mutations in the PAH gene that cause PKU.

Introduction

Classical phenylketonuria (PKU) is an autosomal recessive disease that results from a severe deficiency of the hepatic enzyme phenylalanine hydroxylase (PAH) (Følling 1934). The enzyme normally catalyzes the oxidation of phenylalanine to tyrosine, which is the major metabolic pathway of phenylalanine. This lack of PAH activity causes persistent hyperphenylalaninemia and minor metabolic pathways for phenylalanine become overutilized. Clinical symptoms of the disorder are severe and result in permanent mental retardation in untreated children. The prevalence of PKU among Caucasians is about 1 in 10,000 with a carrier frequency of 1 in 50 (for review, see Scriver and Clow 1980).

Since the enzyme is hepatic and not expressed in amniocytes, there is no biochemical test for in utero detection of PKU. To solve the problem of prenatal diagnosis, molecular approaches were utilized to examine the genetic variation in the PAH gene and the association of this variation with PKU (Woo et al. 1983; Lidsky et al. 1985; DiLella et al. 1986). A full-length cDNA clone of the human PAH gene that confers full enzymatic activity after transfection in mammalian cells was obtained (Kwok et al. 1985; Ledley et al. 1985), and 10 RFLPs at the PAH locus on human chromosome 12q22-24.1 were detected (Lidsky et al. 1984, 1985).

A total of 12 RFLP haplotypes within the PAH locus have been identified in Denmark, and mutant alleles have been observed in association with nine of these haplotypes (Chakraborty et al. 1987). Since there is strong evidence that RFLP haplotypes are in linkage disequilibrium with specific mutant alleles in β-thalassemia (Orin and Kazazian 1984) and in PKU (DiLella et al. 1986, 1987), the observation would suggest that there may be an array of different mutant PAH alleles causing PKU. To estimate the probable extent of mutant PAH alleles among Caucasians, the distribution of RFLP haplotypes was determined in nuclear PKU families from two additional European countries representing different geographic regions in Europe.

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Material and Methods

DNA Analysis

Genomic DNA was prepared from leukocytes isolated from venous blood of nuclear PKU families with at least one affected child. Clinical criteria of the probands as affected individuals and parents as heterozygotes were reported previously elsewhere (Güttler 1980). All families were Caucasian and had no history of consanguinity. The DNA samples were digested with the seven restriction enzymes that yield RFLPs (*Bgl*II, *Pvu*II, *Eco*RI, *Msp*I, *Xmn*I, *Hin*dIII, and *Eco*RV). The digested DNA was fractionated on 1% agarose gels and was transferred to nitrocellulose filters (Lidsky et al. 1985). The probe was the recombinant plasmid phPAH 247 (Kwok et al. 1985) which contains a 2.4-kb human cDNA insert encoding the entire PAH mRNA.

DNA Polymorphism Analysis

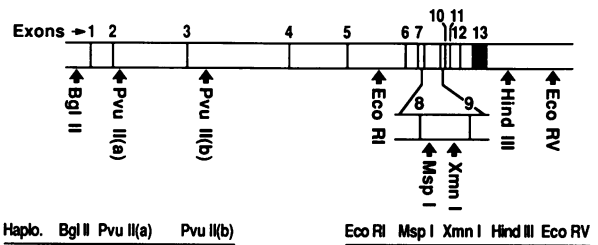
Haplotypes were assigned by determining the presence or absence of the eight polymorphic sites in the PAH gene as described previously elsewhere (Lidsky et al. 1985). Since each patient is an obligate heterozygote for a PKU allele, the haplotypes of four independent chromosomes (two normal and two mutant) from the parents in each family were determined. Unambiguous determination of parental haplotypes was possible in 33 families from Denmark, 19 in Switzerland, and 17 in Scotland. The allele and haplotype frequencies for normal and mutant chromosomes were compared using a χ^2 contingency test. Linkage disequilibria were calculated from the observed haplotype frequencies by using the square root of the χ^2 measure proposed by Morton and Wu (1987). In the two-by-two case this measure is identical to the delta value used in our earlier analysis of the PAH locus (Chakraborty et al. 1987). Separate computations for PKU and non-PKU haplotypes were performed.

Results

Haplotype Frequencies of Normal and Mutant PAH Genes

Eight polymorphic restriction-enzyme sites at the PAH locus were analyzed from the parental chromosomes in 22 and 23 nuclear families from Switzerland and Scotland, respectively. Eighty-one percent of the Swiss families and 65% of the Scottish families in this study were completely informative for determining parental haplotypes. The total of 22 distinct RFLP haplotypes associated with normal and with mutant PAH genes are represented in figure 1.

Restriction Map and RFLP Haplotypes at the PAH Locus



Haplo.	Bgl II	Pvu II(a)	Pvu II(b)	Eco RI	Msp I	Xmn I	Hind III	Eco RV
1	-	+	-	-	+	-	-	-
2	-	+	-	-	+	-	+	+
3	-	+	-	+	-	+	-	-
4	-	+	-	+	-	+	+	+
5	+	-	+	+	+	-	-	+
6	+	-	+	+	+	-	-	-
7	+	-	-	+	-	+	-	-
8	-	+	-	+	+	-	-	+
9	+	+	-	+	+	-	-	+
10	-	+	-	+	+	-	-	-
11	+	-	-	+	+	-	-	+
12	-	+	-	-	+	-	=	+
14	-	-	-	+	+	-	-	-
15	-	-	-	+	+	-	-	+
19	-	+	-	+	-	+	-	+
20	-	-	+	+	+	-	-	-
22	-	+	-	+	+	-	=	+
23	+	-	+	+	-	+	-	-
24	-	+	-	-	+	-	+	-
25	-	+	-	-	+	-	=	-
26	+	+	-	-	+	-	-	-
27	-	+	-	-	+	-	-	+

Figure 1 Restriction map of the human PAH locus, showing the eight polymorphic restriction sites and the definition of various haplotypes. A plus sign (+) indicates the presence of the restriction site; a minus sign, (−) indicates the absence of the site; an equals sign (=) represents the 4.4-kb *Hin*dIII allele. The numerical designation of each haplotype is consistent with a recently collated table of 43 RFLP haplotypes of the human PAH locus that were observed in Europe (Woo 1988). Haplotypes 15, 22, and 24–26 are not present in the three countries reported here but were detected in other European regions.

Previous studies (Chakraborty et al. 1987) established that the relative frequencies of different haplotypes were significantly different among PKU allele–bearing and normal allele–bearing chromosomes in the Danish population. In brief, haplotypes 1 and 4 were found to be very common among normal and PKU chromosomes, while haplotypes 2 and 3 are very common among mutant chromosomes and very rare among normal chromosomes. Tables 1 and 2 extend this observation to Switzerland and Scotland. The frequencies of haplotype 1 and 4 remain high among both normal and mutant chromosomes; these frequencies are .62 and .43, respectively. Conversely, the frequency of haplotypes 2 and 3 accounts for .39 of all mutant chromosomes and for only .08 of all normal chromosomes. However, there is no unique haplotype that is characteristic of PKU chromosomes.

Table 1

Haplotype Frequencies of Normal Chromosomes

Haplotype	No. (frequency) of Haplotypes of Normal Chromosomes			
	Denmark	Switzerland	Scotland	Total
1	23(.35)	15(.42)	10(.32)	48(.36)
2	3(.05)	2(.06)	1(.03)	6(.05)
3	2(.03)	0	2(.06)	4(.03)
4	21(.32)	9(.25)	4(.13)	34(.26)
5	7(.11)	4(.11)	3(.10)	14(.11)
6	0	0	1(.03)	1(.01)
7	7(.11)	4(.11)	3(.10)	14(.11)
8	1(.02)	0	3(.10)	4(.03)
9	0	0	0	0
10	1(.02)	0	0	1(.01)
11	1(.02)	0	0	1(.01)
12	0	0	0	0
14	0	0	1(.03)	1(.01)
15	0	0	0	0
19	0	1(.03)	1(.03)	2(.02)
20	0	0	1(.03)	1(.01)
22	0	0	0	0
23	0	1(.03)	0	1(.01)
24	0	0	0	0
25	0	0	0	0
26	0	0	0	0
27	0	0	1(.03)	1(.01)
Total	66	36	31	133

In the sample of the Danish population, nine different haplotypes are associated with the normal alleles. Of these nine haplotypes, 88% of the normal alleles are confined to only four haplotypes. Similar results were found when this study was extended to Switzerland and Scotland (table 1). The normal alleles are represented by 19 haplotypes. Again, only four haplotypes account for over 84% of the normal alleles. It is interesting that the same four haplotypes (haplotypes 1, 4, 5, and 7) represent the vast majority of normal alleles in each country.

Fifteen different haplotypes were observed for the mutant alleles in Denmark, Switzerland, and Scotland (table 2). As previously reported, of the nine haplotypes associated with mutant PAH genes in Denmark, 90% are confined to haplotypes 1–4. Similarly, haplotypes 1–4 account for 84% of the mutant PAH genes in Switzerland and for 63% of those in Scotland.

Haplotype Distribution of Normal and Mutant PAH Genes

χ^2 Analysis was performed to determine whether the distribution of the most common haplotypes among normal alleles differed between Denmark, Switzerland, and Scotland. A 3 × 5 contingency table was used to obtain a χ^2 statistic for haplotypes 1, 4, 5, and 7, combining the remaining rare haplotypes into the category of "other." The χ^2 obtained was 11.24 with 8 df ($.10 < P < .250$). This value is not significant and suggests that the haplotype distribution of normal PAH genes does not differ significantly between Denmark, Switzerland, and Scotland.

Unlike the normal genes, haplotypes 1–4 and "other" yield a significant χ^2 statistic for the mutant PAH genes. The χ^2 is 31.9 with 8 df ($P < .005$). This highly significant value suggests that, between Denmark, Switzerland, and Scotland, there is considerable variation in the distribution of haplotypes associated with PKU allele–bearing chromosomes.

Allele Frequencies of Normal and Mutant PAH Genes

χ^2 Analysis was used to determine whether the allele frequencies of the normal PAH genes differed significantly between the three European countries. Table 3 shows that no χ^2 statistic obtained for normal genes shows a significant difference between observed and expected allele frequencies for any of the eight poly-

Table 2

Haplotype Frequencies of PKU Chromosomes

Haplotype	No. (frequency) of Haplotypes of PKU Chromosomes			
	Denmark	Switzerland	Scotland	Total
1	12(.18)	19(.50)	10(.30)	41(.30)
2	13(.20)	4(.11)	3(.09)	29(.15)
3	25(.38)	2(.05)	6(.18)	33(.24)
4	9(.14)	7(.18)	2(.06)	18(.13)
5	0	0	1(.03)	1(.01)
6	2(.03)	2(.05)	0	4(.03)
7	1(.02)	0	0	1(.01)
8	0	1(.03)	1(.03)	2(.01)
9	1(.02)	0	1(.03)	2(.01)
10	0	0	0	0(.01)
11	1(.02)	0	0	1(.01)
12	2(.03)	2(.05)	1(.03)	5(.04)
14	0	0	1(.03)	1(.01)
15	0	0	1(.03)	1(.01)
19	0	0	0	0
20	0	0	2(.06)	2(.01)
22	0	1(.03)	0	1(.01)
23	0	0	0	0
24	0	0	1(.03)	1(.01)
25	0	0	1(.03)	1(.01)
26	0	0	1(.03)	1(.01)
27	0	0	1(.03)	1(.01)
Total	66	38	33	137

Table 3

Allele Frequencies of Normal Chromosomes

Enzyme and Allele	No. (frequency) of Alleles of Normal Chromosomes				χ^2	df	*P*
	Denmark	Switzerland	Scotland	Total			
*Bgl*II:							
3.6	51(.77)	27(.75)	24(.77)	102(.77)	.79	2	n.s.
1.7	15(.23)	9(.25)	7(.23)	31(.23)			
*Pvu*IIa							
19.6	15(.23)	10(.28)	9(.29)	34(.26)	.57	2	n.s.
6.0	51(.77)	26(.72)	22(.71)	99(.74)			
*Pvu*IIb:							
11.5	59(.90)	31(.86)	26(.84)	116(.87)	.63	2	n.s.
9.1	7(.10)	5(.14)	5(.16)	17(.13)			
*Eco*RI:							
17.0	26(.39)	17(.47)	12(.39)	55(.41)	.71	2	n.s.
11.0	40(.61)	19(.53)	19(.61)	78(.59)			
*Msp*I:							
23.0	30(.46)	15(.42)	10(.32)	55(.41)	1.52	2	n.s.
19.0	36(.54)	21(.58)	21(.68)	78(.59)			
*Xmn*I:							
9.4	36(.54)	21(.58)	21(.68)	78(.59)	1.52	2	n.s.
6.5	30(.46)	15(.42)	10(.32)	55(.41)			
*Hin*dIII:							
4.4	0	0	0	0	4.11	4	n.s.
4.2	42(.64)	25(.69)	26(.84)	93(.70)			
4.0	24(.36)	11(.31)	5(.16)	40(.30)			
*Eco*RV:							
30.0	33(.50)	20(.56)	18(.58)	71(.53)	.65	2	n.s.
25.0	33(.50)	16(.44)	13(.42)	62(.47)			

NOTE.—n.s. = not significant.

morphisms among the three countries. This suggests that allele frequencies of normal PAH genes remain relatively constant between Denmark, Switzerland, and Scotland. Since a significant variation between the three countries was observed with respect to haplotype distribution of mutant PAH genes, a difference in allele frequencies might also be expected. Accordingly, three enzymes show considerable allelic frequency variation between Denmark, Switzerland, and Scotland (table 4). *Eco*RI, *Msp*I, and *Xmn*I give significant χ^2 statistics. The χ^2 for *Eco*RI is 6.18 with 2 df ($.125 < P < .05$). *Msp*I and *Xmn*I both have χ^2 values of 12.3 with 2 df ($.005 < P < .01$). Equal χ^2 statistics are generated because *Msp*I and *Xmn*I are in complete linkage disequilibrium. This is consistent with the small map distance separating these sites and also reflects that no recombination has occurred between these two loci.

Linkage Disequilibria among RFLP Sites at the PAH Locus

Table 5 shows standardized linkage disequilibrium coefficients used to study the relationship between specific RFLP sites and PKU. The linkage disequilibria were computed separately for the normal and PKU chromosomes (table 5). The method of Morton and Wu (1988) is applicable to any number of alleles, so the *Hin*dIII locus, with three alleles (4.0-, 4.2-, and 4.4-kb fragments), was analyzed as three separate alleles—rather than as two alleles (4.0 and 4.2 + 4.4), as in earlier work (Chakraborty et al. 1987). As expected, table 5 shows that the RFLP sites that are in close proximity are always significantly associated (see values shown in italics). The distantly located RFLP sites are in random association in all cases. These observations hold for both the normal and PKU chromosomes. Additionally, the RFLP sites break into a 5′ and a 3′ cluster. The magnitudes of disequilibrium values within each cluster are consistent with the map distance between sites.

Linkage Disequilibrium between RFLP Sites and PKU

Table 5 also shows linkage disequilibrium values for each RFLP and PKU. Again, because the *Hin*dIII poly-

Table 4

Allele Frequencies of PKU Chromosomes

Enzyme and Allele	No. (frequency) of Alleles of PKU Chromosomes				χ^2	df	*P*
	Denmark	Switzerland	Scotland	Total			
*Bgl*II:							
3.6	61(.92)	36(.95)	30(.91)	127(.93)	.40	2	n.s.
1.7	5(.08)	2(.05)	3(.09)	10(.07)			
*Pvu*IIa							
19.6	4(.06)	2(.05)	5(.15)	11(.08)	3.01	2	n.s.
6.0	62(.94)	36(.95)	28(.85)	126(.92)			
*Pvu*IIb:							
11.5	64(.97)	36(.95)	30(.91)	130(.95)	1.67	2	n.s.
9.1	2(.03)	2(.05)	3(.09)	7(.05)			
*Eco*RI:							
17.0	27(.41)	25(.66)	18(.55)	70(.51)	6.18	2	$.025 < P < .05$
11.0	39(.59)	13(.34)	15(.45)	67(.49)			
*Msp*I:							
23.0	35(.53)	9(.24)	8(.24)	52(.37)	12.30	2	$.005 < P < .01$
19.0	34(.47)	29(.76)	25(.76)	85(.62)			
*Xmn*I:							
9.4	31(.47)	29(.76)	25(.76)	85(.62)	12.30	2	$.005 < P < .01$
6.5	35(.53)	9(.24)	8(.24)	52(.37)			
*Hin*dIII:							
4.4	2(.03)	3(.08)	2(.06)	7(.05)	3.96	4	n.s.
4.2	41(.62)	24(.63)	25(.76)	90(.66)			
4.0	23(.35)	11(.29)	6(.18)	40(.29)			
*Eco*RV:							
30.0	39(.59)	23(.61)	21(.64)	83(.61)	.19	2	n.s.
25.0	27(.41)	15(.39)	12(.36)	54(.39)			

Note.—n.s. = not significant.

morphism has three alleles, values were computed separately according to the method of Morton and Wu. Our results give a value of .31 for *Hin*dIII, where values >.16 are significantly different from .00 ($P < .01$). No other RFLP approaches significance.

Discussion

The main objectives of this study were to (1) estimate the extent of mutant PAH alleles among Caucasians, (2) compare the haplotype distribution among normal and PKU-bearing chromosomes, (3) compare the haplotype distributions and allele frequencies between European populations, (4) compare standardized pairwise linkage disequilibrium coefficients for each RFLP with the physical PAH RFLP map, and (5) determine whether there is any linkage disequilibrium between specific RFLPs and PKU. A sample size of $N = 133$ for normal chromosomes and of $N = 137$ for PKU chromosomes was generated by analyzing 87 nuclear Caucasian families from Denmark, Switzerland, and Scotland. Approximately 77% of the families in this study were completely informative for haplotype analysis. It should be noted that a family not informative for haplotype analysis is not necessarily uninformative for prenatal diagnosis. Successful prenatal diagnosis of PKU requires only that the family be informative for one enzyme. Daiger et al. (1986) earlier demonstrated that use of the PKU haplotypes based on RFLPs would establish disease status in approximately 87% of siblings at risk. Likewise, when this analysis is extended to the Swiss and Scottish populations, prenatal diagnosis is possible in approximately 86% and 78% of the families analyzed, respectively. Collectively, prenatal diagnosis is possible in 85% of the families studied.

In the Danish population it was previously observed that the haplotype frequencies for normal and PKU chromosomes are markedly different (Chakraborty et

Table 5

Standardized Linkage Disequilibria among RFLP Sites at the PAH Locus, Estimated from Haplotype Frequencies of Normal and PKU-bearing Chromosomes

	*Bgl*II	*Pvu*IIa	*Pvu*IIb	*Eco*RI	*Msp*I	*Xmn*I	*Hin*dIII	*Eco*RV
RFLPs on normal chromosomes (N = 133):[a]								
*Pvu*IIa	.92							
*Pvu*IIb	.41	.44						
*Eco*RI.....	.21	.23	.10					
*Msp*I	.01	.00	.08	.50				
*Xmn*I	.01	.00	.08	.50	1.00			
*Hin*dIII....	.13	.14	.06	.12	.34	.34		
*Eco*RV....	.00	.00	.08	.33	.10	.10	.49	
RFLPs on PKU chromsomes (N = 137):[b]								
*Pvu*IIa	.41							
*Pvu*IIb	.33	.62						
*Eco*RI.....	.05	.09	.06					
*Msp*I	.03	.03	.03	.64				
*Xmn*I	.03	.03	.04	.64	1.00			
*Hin*dIII....	.02	.02	.01	.01	.02	.02		
*Eco*RV....	.00	.00	.01	.00	.00	.00	.05	
PKU vs. normal RFLPs (N = 270):[c]								
PKU.....	.05	.05	.02	.01	.00	.00	.31	.01

NOTE.—RFLPs are shown in chromosomal order.

[a] Values > .26 (underlined) are significantly different from .00 ($P < .01$).

[b] Values > .25 (underlined) are significantly different from .00 ($P < .01$).

[c] Values > .16 (underlined) are significantly different from .00 ($P < .01$).

al. 1987). This conclusion is supported by data from the Swiss and Scottish populations. Overall, haplotypes 1–4 represent 80% of the normal chromosomes and 82% of the PKU chromosomes. Haplotypes 1 and 4 are relatively common among both normal and mutant chromosomes. However, haplotypes 2 and 3 represent 40% of the PKU alleles and only 8% of the normal alleles. Since specific mutations have been shown to be in strong linkage disequilibrium with certain haplotypes in β-thalassemia (Orkin and Kazazian 1984), a systematic approach to identify the most prevalent PKU mutations was begun. Previous RFLP haplotype analysis of the PAH locus in Denmark showed different mutations causing PKU to be associated with different RFLP haplotypes. As an example, an amino acid substitution (Arg→Trp) at residue 408 was found to be in linkage disequilibrium with RFLP haplotype 2 (DiLella et al. 1987). Additionally, a mutation at the 5′ splice site of intron 12 was found to be uniquely associated with RFLP haplotype 3 (DiLella et al. 1986). Both mutations are associated with a specific haplotype and are not found in mutant alleles of other haplotypes. Oligonucleotide hybridization with probes specific for each mutation has shown complete concordance with these mutations in the Danish, Swiss, and Scottish populations (data not shown). Thus, the absolute association between the two PKU mutations and specific RFLP haplotypes has been extended to three European countries. Since additional haplotypes are associated with PKU in these populations, it is likely there are additional PKU mutations involved.

In this study, we have compared the haplotype distributions and allele frequencies in Denmark, Switzerland, and Scotland. Neither the haplotype distributions nor the allele frequencies of normal chromosomes change significantly between these three European populations. However, for the PKU allele–bearing chromosomes, both the haplotype distributions and allele frequencies show significant variation between the three countries, and similar observations have recently been made in Germany by three independent studies (Aulehla-Scholz et al. 1988; Lichter-Konecki et al. 1988; Reiss et al. 1988). This suggests that PKU may be caused by multiple mutations that occurred on various ethnic backgrounds.

The standardized linkage disequilibrium values determined for each RFLP support the previously deter-

mined RFLP map of the PAH locus (Chakraborty et al. 1987). The sites segregate into a 5′ and a 3′ cluster. Sites within each cluster are in close proximity and are in linkage disequilibrium in all cases. No significant disequilibrium between the two clusters is observed in any instance. Previously, more distantly located RFLP sites were found to be in random association, except in two instances, the *Eco*RI:*Bgl*II and the *Hin*dIII:*Pvu*IIa pairs. In this study, the sample size is increased from $N = 132$ to $N = 270$, and this nonrandom association between distantly located RFLPs is no longer observed.

No close association of PKU with a particular RFLP was observed previously in the Danish population. This is consistent with multiple PKU mutations arising on independent, unrelated chromosomes. However, our combined data show a significant association between *Hin*dIII and PKU not previously observed. This value must be interpreted with caution, since the infrequently represented 4.4-kb *Hin*dIII allele ($N = 7$) is present only on PKU allele–bearing chromosomes while the 4.2-kb ($N = 218$) and 4.0-kb alleles ($N = 79$) are found on both normal and mutant chromosomes. However, the 4.4-kb *Hin*dIII allele is not uniquely associated with PKU, since this allele has previously been observed to segregate with normal chromosomes (Speer et al. 1986).

The characterization of the mutations associated with haplotypes 1 and 4 would theoretically account for 82% of all the mutant chromosomes in Denmark, Switzerland, and Scotland. If these four haplotypes account for an equally large percentage of PKU chromosomes in other Caucasian populations, carrier screening for PKU by oligonucleotide hybridization analysis would be possible in families with no previous history of PKU.

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