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ARTICLE

The *ABCA4* 2588G > C Stargardt mutation: single origin and increasing frequency from South-West to North-East Europe

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Inherited retinal dystrophies represent the most important cause of vision impairment in adolescence, affecting approximately 1 out of 3000 individuals. Mutations of the photoreceptor-specific gene *ABCA4 (ABCR)* are a common cause of retinal dystrophy. A number of mutations have been repeatedly reported for this gene, notably the 2588G > C mutation which is frequent in both patients and controls. Here we ascertained the frequency of the 2588G > C mutation in a total of 2343 unrelated random control individuals from 11 European countries and 241 control individuals from the US, as well as in 614 patients with STGD both from Europe and the US. We found an overall carrier frequency of 1 out of 54 in Europe, compared with 1 out of 121 in the US, confirming that the 2588G > C *ABCA4* mutation is one of the most frequent autosomal

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recessive mutations in the European population. Carrier frequencies show an increasing gradient in Europe from South-West to North-East. The lowest carrier frequency, 0 out of 199 (0%), was found in Portugal; the highest, 11 out of 197 (5.5%), was found in Sweden. Haplotype analysis in 16 families segregating the 2588G > C mutation showed four intragenic polymorphisms invariably present in all 16 disease chromosomes and sharing of the same allele for several markers flanking the *ABCA4* locus in most of the disease chromosomes. These results indicate a single origin of the 2588G > C mutation which, to our best estimate, occurred between 2400 and 3000 years ago.
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Introduction

Inherited retinal dystrophies represent the most important cause of vision loss in adolescence, affecting approximately 1 out of 3000 individuals. Mutations in a photoreceptor-specific ATP-binding cassette transporter gene, *ABCA4*, have been implicated in patients with autosomal recessive Stargardt disease (STGD),[1–10] cone-rod dystrophy[6,10–12] and retinitis pigmentosa.[11,13,14] Moreover, heterozygous *ABCA4* mutations have been associated with an increased susceptibility to age-related macular dystrophy, a multifactorial disorder that frequently affects the elderly.[15–17]

To explain how mutations in a single gene give rise to a spectrum of different phenotypes, a model has been proposed that correlates the severity of the retinal dystrophy with the severity of the mutations and the residual activity of the ABCR protein.[4,5,11,13,18,19]

ABCA4 is specifically expressed in cone and rod photoreceptor outer segments,[1,20] where it is thought to act as a *N*-retinylidene-phosphatidylethanolamine flippase,[21,22] moving all-*trans*-retinal from the lumenal to the cytosolic side of the discs.

Mutation analysis has shown a very high allelic heterogeneity for the *ABCA4* gene. Most of the known mutations account only for a few cases of STGD or other *ABCA4*-associated retinal disorders. However, a few mutations showed a relatively high frequency and have been repeatedly reported in European and American studies. In particular, a 2588G > C mutation was identified heterozygously in 15 out of 40 (37.5%) Dutch and German patients and in 9 out of 311 (2.9%) control individuals from the Netherlands.[5] The mutation was shown to be in linkage disequilibrium with another DNA variant, 2828G > A, suggesting a founder effect.[5]

Because of its high frequency in the population, the pathological significance of the 2588G > C variant has been disputed. Maugeri *et al*[5] showed that the mutation results in two RNA products which are equally abundant in lymphoblastoid cells. The mutant transcripts encode an ABCR protein with a deletion of glycine 863, due to aberrant splicing, or a substitution of the same glycine to alanine. Recently, functional studies[23] clearly demonstrated that both the ΔG863 and G863A variant impair ABCR protein function.

To investigate the occurrence of the 2588G > C allele in different parts of Europe and find clues on the origin of the mutation, we ascertained its frequency in 11 European countries and the US, and performed haplotype analysis using 16 families segregating the 2588G > C *ABCA4* mutation.

Materials and methods

Materials

A total of 2343 unrelated random control individuals from 11 European countries, ie Belgium, Denmark, Estonia, France, Germany, Italy, the Netherlands, Portugal, Spain, Sweden and the UK, and 241 control individual from the US were included in the study. Moreover, we studied a total of 614 patients with STGD from Denmark, France, Germany, the Netherlands, UK and US. The ethnic background could not be ascertained for all controls and patients included in the study, so that presence of (a small number of) recently immigrated individuals can not be ruled out.

Haplotype analysis was performed using 16 families with STGD segregating the 2588G > C *ABCA4* mutation. Twelve families were of Dutch origin, three were from Germany and one from Sweden.

Methods

The presence of the 2588G > C *ABCA4* mutation was assessed by each participating center using one of the available standardised methodologies. These included allele-specific oligonucleotide (ASO) hybridisation analysis,[24] the amplification refractory mutation system polymerase chain reaction technique[25] or direct sequence analysis of exon 17 of the *ABCA4* gene.

Microsatellite markers distributed over approximately a 7 Mb interval spanning the *ABCA4* locus and single nucleotide polymorphisms (SNPs) in the coding region of the *ABCA4* gene were used for haplotype analysis. Microsatellite markers included (from 1pter to cen): D1S2804 (AFMb363xf9), D1S406 (UT2069), D1S2868 (AFMa051wg9),

D1S2849 (AFM350tg9), D1S236 (AFM205ta11), D1S2664 (AFM164wh2), D1S497 (AFM331vb1) and D1S420 (AFM199xb6). The size of the alleles was assessed using a control DNA sequence from bacteriophage M13mp18 as a marker. Sequence reaction was performed with the DNA Sequencing Kit Sequenase Version 2.0 (Amersham). Intragenic polymorphisms were assessed by ASO hybridisation analysis, direct sequencing or restriction analysis.

The degree of linkage disequilibrium was assessed employing the parameter δ as defined by Risch *et al*.[26] δ represents an estimate of the proportion of disease chromosomes carrying the ancestral associated allele, based on the assumption that a disease chromosome not bearing an original associated allele (either due to a mutation or through recombination) may still carry that allele with probability p_N.[26]

To estimate the age of the mutation, we used a method which assumes that all alleles coalesce close to the original founder.[26] This procedure is likely to bias the estimated age of origin such that it is estimated younger than it actually is. We used here a theoretical estimate for θ (1 Mb=1 cM), which implies that the unknown evolutionary history of the haplotype has a substantial, but not easily evaluated, influence on the error of the estimated age.

Results

In total, 43 out of 2343 European control individuals were found to carry the *ABCA4* 2588G>C mutation heterozygously (carrier frequency=1 out of 54), compared to 2 out of 241 samples in the US (carrier frequency=1 out of 121). The distribution of the frequencies in each European country is reported in Table 1. Carrier frequencies tend to increase in Europe from South-West to North-East (Table 1 and Figure 1). The lowest carrier frequency was detected in Portugal (0 out of 199) while in Sweden 11 out of 197 control individuals had the 2588G>C *ABCA4* mutation. Frequencies in patients with STGD, only available for some European countries, show a similar distribution, with a relatively high incidence in the Netherlands and Denmark and a lower frequency in France (Table 1).

Haplotype analysis in 16 families segregating the 2588G>C mutation, showed four intragenic polymorphisms, ie 2828G>A (R943Q), 4203C>A (P1401P), 5603A>T

Figure 1 Frequencies of the 2588C *ABCA4* allele (%) in European countries (in grey).

Table 1 Occurrence of the 2588G>C *ABCA4* mutation in 11 European countries and the US

	Controls			*STGD*[a]	
	No. 2588C alleles/No. chromosomes (%)		*Carrier frequency*	*No. 2588C alleles/No. chromosomes (%)*	
Sweden	11 out of 394	(2.8)	1 out of 18		
The Netherlands	9 out of 622	(1.4)[b]	1 out of 35	22 out of 126	(17.5)
Estonia	4 out of 390	(1.0)	1 out of 49		
Denmark	2 out of 200	(1.0)	1 out of 50	10 out of 98	(10.2)
Italy	4 out of 400	(1.0)	1 out of 50		
Germany	5 out of 672	(0.7)	1 out of 67	21 out of 310	(6.8)
France	3 out of 434	(0.7)	1 out of 72	7 out of 254	(2.8)
UK	2 out of 352	(0.6)[c]	1 out of 88	5 out of 140	(3.6)[c]
Belgium	2 out of 406	(0.5)	1 out of 102		
Spain	1 out of 418	(0.2)[d]	1 out of 209		
Portugal	0 out of 398	(0.0)	0 out of 199		
Total Europe	43 out of 4686	(0.9)	1 out of 54	65 out of 928	(7.0)
US	2 out of 482	(0.4)	1 out of 121	11 out of 300	(3.7)[e]

[a]Frequencies were calculated only when more than 80 chromosomes were analysed. [b–e]Data previously published in ref. 5, 6, 10 and 4, respectively.

Marker	Haplotypes															
D1S2804	203	205	211	207	205	211	207	207	211	209	209	209	207	205	209	209
D1S406	234	222	222	230	230/222	222	230	234	222	222	218	222	222	230	222	226
D1S2868	168	166	164	168	168	164	168	168	164	164	164	164	164	168	164	164
D1S2849	204	202	204	206	202	204	202	206	204	204	204	204	204	204	204	204
ABCA4 5682G>C L1894L	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
ABCA4 5603A>T N1868I	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
ABCA4 4203C>A P1401P	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
ABCA4 2828G>A R943Q	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
ABCA4 2588G>C ΔG863/G863A →	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
D1S236	208	208	208	234	208	208	208	230	208	208	208	208	234	208	208	208
D1S2664	258	266	264	258	266	264/266	266	264	266	266	266	266	268	262	266	266
D1S497	295	273	281	281	281	295	295	281	281	293	281	275	285	281	281	285
D1S420	224	226	224	232	218/224	230	224	232	224	218	224/226	230	226	224	222	226
Family no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Country	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	S	D	D	D

Figure 2 Haplotypes containing the 2588C allele in 16 patients with STGD from the Netherlands (NL), Germany (D) and Sweden (S). Shared alleles, thought to correspond to the ancestral disease haplotype, are depicted in grey.

(N1868I) and 5682G > C (L1894L), invariably present in all 16 disease chromosomes, whether from the Netherlands, Germany or Sweden (Figure 2). Moreover, co-occurrence of the 2588G > C mutation and the 2828G > A change was observed in all patients with STGD, 22 from the Netherlands, 22 from Germany, six from France, five from UK, one from Spain and 11 from US, in which both variants were investigated (data not shown).

Seven out of 16 disease chromosomes shared a common haplotype for the distal markers D1S406, D1S2868 and D1S2849 (Figure 2). This haplotype was also shared by two control chromosomes carrying the intragenic polymorphisms 4203C > A, 5603A > T and 5682G > C (Figure 3). Nine out of 16 disease chromosomes exhibit the same alleles for the proximal markers D1S236 and D1S2664 (Figure 2).

The degree of linkage disequilibrium (δ) at each locus was calculated over the frequency of the most common allele in the 16 disease chromosomes and the frequency of the same allele in 30 ethnically matched control chromosomes (Table 2). The value of δ is maximum for the intragenic polymorphisms (δ=1.00). Among microsatellite markers, δ is the highest for the proximal locus D1S236 and for the distal locus D1S2849. Although δ is not a very reliable indicator of genetic distance, lower values for the degree of linkage disequilibrium across loci reflects among other things the effect of recombinations, and can with caution be used for mapping of loci. The physical order of the markers reported in Figure 2 is suggested by haplotype sharing and values of δ, and is in agreement with the *ABCA4* region in the genome working-draft sequence (August 6, 2001).

The number of generations since the appearance of the 2588G > C mutation, estimated for the nearest marker D1S236, is 118. Assuming a generation length between 20 and 25 years, this would suggest an age for the mutation between 2400 and 3000 years.

Discussion

This study confirms the high frequency of the 2588G > C *ABCA4* mutation in many European countries. The overall carrier frequency (1 out of 54) points to the 2588G > C *ABCA4* mutation as being one of the most frequent autosomal recessive disease mutations in the European population. A comparable carrier frequency has been recently reported for the 35delG mutation in the connexin 26 (*GJB2*) gene (1 out of 51.1),[27] whose heterozygote frequencies were significantly higher in the Mediterranean countries. In contrast, the 2588G > C *ABCA4* mutation showed increasing heterozygote frequencies from South-West to North-East Europe (Figure 1, Table 1). A similar but distinct distribution of frequencies has been described in Europe for the ΔF508 mutation in the cystic fibrosis gene, *ABCC7-CFTR*, whose gradient has a South-East to North-West orientation,[28] and thus does not overlap the gradient of the *ABCA4* mutation.

The observed cline in the frequency of the 2588G > C allele in Europe could result from migratory fluxes, coupled with a founder effect. For alleles descending from a single ancestor, slow diffusion into other populations is the likely course of history. It is difficult however to prove this from a statistical standpoint. If the mutation had been seeded with the same

Marker	Haplotypes																													
D1S2804	211	207	211	209	205	205	199	213	211	?	205	215	203	205	205	205	205	205	205	205	205	207	205	199	211	205	209	205	205	199
D1S406	242	230	234	226	222	234	222	?	234	230	226	234	226	230	226	222	226	234	222	222	242	234	230	222	242	234	234	234	230	226
D1S2868	164	164	168	168	168	170	164	170	168	168	168	164	168	170	168	168	164	166	168	168	168	168	166	164	168	164	168	168	168	166
D1S2849	198	208	202	204	202	202	204	198	202	202	200	194	?	204	204	204	206	200	206	200	196	202	202	204	198	200	206	202	204	202
ABCA4 5682	C	G	C	G	C	G	C	C	G	G	C	G	G	G	G	G	G	G	G	G	G	G	G	C	G	C	G	G	C	G
ABCA4 5603	A	A	T	A	A	A	T	T	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	T	A	A	A	A	A	A
ABCA4 4203	C	C	C	C	C	C	A	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	C	C	C	C	C	C
ABCA4 2828	G	G	G	G	G	G	G	G	A	G	G	G	A	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
D1S236	230	212	208	230	230	208	212	208	210	208	212	212	212	230	208	208	208	208	232	212	228	212	230	208	208	208	232	212	228	230
D1S2664	264	264	264	266	266	264	266	258	264/266	264/266	270	266	264	264	264	266	268	258	258	258	264	266	264	262	264	264	266	268	268	266
D1S497	275	281	281	281	281	281	277	281	305	281	295	295	291	291	281	291	281	281	293	293	291	285	291	299	293	281	285	275	273	281
D1S420	224	226	226	224	224	222	222	?	220	226	234	222	224	226	226	226	226	224	?	224/226	224	226	224	226	230	226	222	226	228	226
Haplotype no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Country	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	S	S	D	D	D	D	D	D

Figure 3 Haplotypes in 30 ethnically matched control chromosomes. Rare polymorphic variants at positions 2828, 4203, 5603 and 5682 of the *ABCA4* gene and marker alleles at D1S2849, D1S2868 and D1S406 presumed to be part of the ancestral disease haplotype are depicted in grey.

Table 2 Frequency of the most commom allele in the 16 disease chromosomes from the Netherlands, German and Sweden, frequency of the same allele in 30 ethnically matched control chromosomes, and degree of linkage disequilibrium (δ) at each locus

Marker	*Distance* (kb)[a]	*Allele*	*Frequency*		*Degree of linkage disequilibrium* (δ)[b]
			2588G>C chromosomes (p_D)	*Control chromosomes* (p_N)	
D1S2804	1790	209	0.31	0.07	0.26
D1S406	1650	222	0.53	0.21	0.41
D1S2868	1340	164	0.56	0.23	0.43
D1S2849	1070	204	0.69	0.24	0.59
ABCA4 5682G>C	41	C	1.00	0.30	1.00
ABCA4 5603A>T	41	T	1.00	0.13	1.00
ABCA4 4203C>A	21	A	1.00	0.10	1.00
ABCA4 2828G>A	5	A	1.00	0.07	1.00
D1S236	300	208	0.81	0.37	0.70
D1S2664	1520	266	0.60	0.28	0.44
D1S497	4370	281	0.50	0.40	0.17
D1S420	5240	224	0.36	0.26	0.14

[a]Distance from each marker to the 2588G>C mutation estimated from the genome working-draft sequence (August 6, 2001). [b]$\delta=(p_D-p_N)/(1-p_N)$.

number of founders in each population, genetic drift would still cause considerable differences in frequency between populations.

The highest frequency of the 2588G>C mutation in Sweden could indicate an origin of the mutation somewhere in the North-North Eastern parts of Europe. Nevertheless, the mutation could have originated in a population before its settling in Europe. An estimation of the age of the mutation suggests that this single event could have taken place between 2400 and 3000 years ago. However, dating of mutations should be regarded with extreme caution, as they highly depend on the method of choice and are based on a number of assumptions that are never entirely fulfilled.

Several studies indicated that founder mutations are quite common in the European population.[29–32] The presence of a

carrier advantage has been proposed to explain the high frequency of some of these founder variants.[33] It is difficult to hypothesise a carrier advantage for individuals carrying the 2588G>C *ABCA4* mutation. The *ABCA4* gene showed a high specificity of expression for the photoreceptor cells. Some minor expression of *ABCA4* has been detected also in the kidney and in the brain.[34] This raises the possibility that *ABCA4* could have a second, unknown function in non-ocular tissues.

An intriguing hypothesis suggests the *ABCA4* mutation to be in linkage disequilibrium with a mutation or polymorphism in a neighbouring gene conferring a selective advantage to heterozygous carriers. Interestingly, the glutamate-cysteine ligase, modifier subunit (*GCLM*), is located between 5682G>C and D1S2849 and thus lies very close to *ABCA4*. *GCLM* encodes the light regulatory subunit of the gamma-glutamylcysteine synthetase, the first rate-limiting enzyme in glutathione biosynthesis. Glutathione is an important antioxidant and is ubiquitously expressed in mammalian tissues. Deficiency of gamma-glutamylcysteine synthetase is known to cause haemolytic anaemia.[35]

More simply, genetic drift could account for the high frequency of the 2588G>C *ABCA4* mutation in the European populations.

As mentioned before, functional studies have shown unequivocally a biochemical defect for both the G863A and the ΔG863 variants of the ABCR protein. Nevertheless, a pertinent question is whether any of the four polymorphisms in linkage disequilibrium with the 2588G>C change are necessary to induce a pathological effect or contribute to ABCR dysfunction. All four SNPs are thought not to be pathologic due to their high frequency in the control population.[1,5] Haplotypes of control (Figure 3) and disease chromosomes (data not shown) suggest that the 5682G>C variant is the oldest. The 5603A>T and the 4203C>A changes likely arose in an individual carrying the 5682G>C variant. The 2828G>A variant probably occurred independently and was added to the haplotype containing the other three changes by a recombination event. Finally, the 2588G>C change arose on this haplotype. The 4203C>A and 5682G>C changes represent silent substitutions and most likely do not influence the function of ABCR. In contrast, the 2828G>A and the 5603A>T changes substitute an arginine to a glutamine and an asparagine to an isoleucine, respectively. These potentially could affect the function of the transporter. Interestingly, functional studies[23] showed a small but reproducible decrease in ATPase activity, relative to wild type, for the N1868I (5603A>T) protein. However, we observed a recombination between the 2828G>A and the 4203C>A or between the 4203C>A and the 5603A>T polymorphisms in at least nine out of a total of 44 patients with STGD, for most of whom the phase could not be ascertained (data not shown). This suggests that the haplotype carrying the 2588G>C and the 2828G>A changes but not the 5603A>T variant is disease-causing.

It has been hypothesised that the 2588C allele would represent a mild allele and would not be disease causing in homozygous state.[5] This hypothesis is strengthened by the high frequency of the 2588C allele detected in Sweden in this study. In fact, based on a carrier frequency of 1 out of 18, approximately 1 out of 1300 individuals are expected to be homozygous for the 2588G>C mutation in Sweden. This is in striking contrast with an incidence of STGD that is apparently not higher in this country than in the rest of Europe and presumably close to the incidence of 1 out of 10 000 ascertained in the American population.[36]

In conclusion, in this study we have confirmed the high occurrence of the 2588G>C *ABCA4* mutation overall in Europe and in particular in the Northern European countries, and that this mutation originated from a single mutation event which, to our best estimate, occurred between 2400 and 3000 years ago.

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