Mutations in *ASPH* Cause Facial Dysmorphism, Lens Dislocation, Anterior-Segment Abnormalities, and Spontaneous Filtering Blebs, or Traboulsi Syndrome

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We have previously described a syndrome characterized by facial dysmorphism, lens dislocation, anterior-segment abnormalities, and spontaneous filtering blebs (FDLAB, or Traboulsi syndrome). In view of the consanguineous nature of the affected families and the likely autosomal-recessive inheritance pattern of this syndrome, we undertook autozygosity mapping and whole-exome sequencing to identify *ASPH* as the disease locus, in which we identified two homozygous mutations. *ASPH* encodes aspartyl/asparaginyl β-hydroxylase (ASPH), which has been found to hydroxylate aspartic acid and asparagine residues on epidermal growth factor (EGF)-domain-containing proteins. The truncating and missense mutations we identified are predicted to severely impair the enzymatic function of ASPH, which suggests a possible link to other forms of ectopia lentis given that many of the genes implicated in this phenotype encode proteins that harbor EGF domains. Developmental analysis of *Asph* revealed an expression pattern consistent with the proposed link to the human syndrome. Indeed, *Asph*-knockout mice had a foreshortened snout, which corresponds to the facial abnormalities in individuals with Traboulsi syndrome. These data support a genetic basis for a syndromic form of ectopia lentis and the role of aspartyl hydroxylation in human development.

The eye is a key sensory organ, and the remarkable conservation of its developmental signals across distant species has facilitated the unraveling of much of the molecular underpinning of that developmental process.[1] Remarkably, more than 90% of human genes are expressed at some stage in at least one ocular tissue, which argues for the complex genetic network that controls the development and function of the eye.[2] Not surprisingly, ocular abnormalities are common features of numerous syndromes, and in some instances, the defects can be highly distinctive or pathognomonic. One of the less common malformation syndromes with very distinctive ocular features was first reported in 1995 in a multiplex consanguineous family from the Druze sect in Lebanon.[3] Virtually all affected individuals had dislocated crystalline lenses and anterior-segment abnormalities, in addition to a highly characteristic face with flat cheeks and a beaked nose. Several affected members developed highly unusual nontraumatic conjunctival cysts (filtering blebs) that were presumably caused by abnormal thinning of the sclera. Two other Lebanese families were subsequently reported, and an entry for this syndrome was created in Online Mendelian Inheritance In Man (MIM 601552).[4,5] In the present report, we describe an additional ethnically distinct Saudi Arabian individual with the same syndrome (Figure 1), which we propose should be called Traboulsi syndrome, or FDLAB (facial dysmorphism, lens dislocation, anterior-segment abnormalities, and spontaneous filtering blebs) syndrome. We were able to combine molecular genetic data from this Saudi affected individual and data from two previously published Lebanese affected individuals from distinct families to determine that mutations in the gene encoding an enzyme that hydroxylates aspartic acid and asparagine residues of epidermal growth factor (EGF)-domain-containing proteins are the likely cause of the syndrome.

Individual 1 (VII:1 in Figure 1) is a 19-year-old Saudi female who first noticed visual difficulties at the age of 10 years. She is the first child of consanguineous parents, and her younger siblings are unaffected by history (Figure 1). She has no health issues except for her ocular problems. Referral was for evaluation following the implantation of scleral-fixated intraocular lenses in both eyes within the preceding year to treat aphakia after the removal of an anteriorly dislocated lens in the right eye and spherophakia in the left eye. Her facial features were consistent with FDLAB syndrome (Figure 1). Best-corrected visual acuity was 20/25 in both eyes. Slit-lamp examination revealed stable intraocular implants and filtering blebs and patchy iris atrophy in both eyes (Figure 1). Retinal examination was within normal limits.

Given the parental consanguinity of individual 1, we hypothesized that the causal mutation is a recessive mutation

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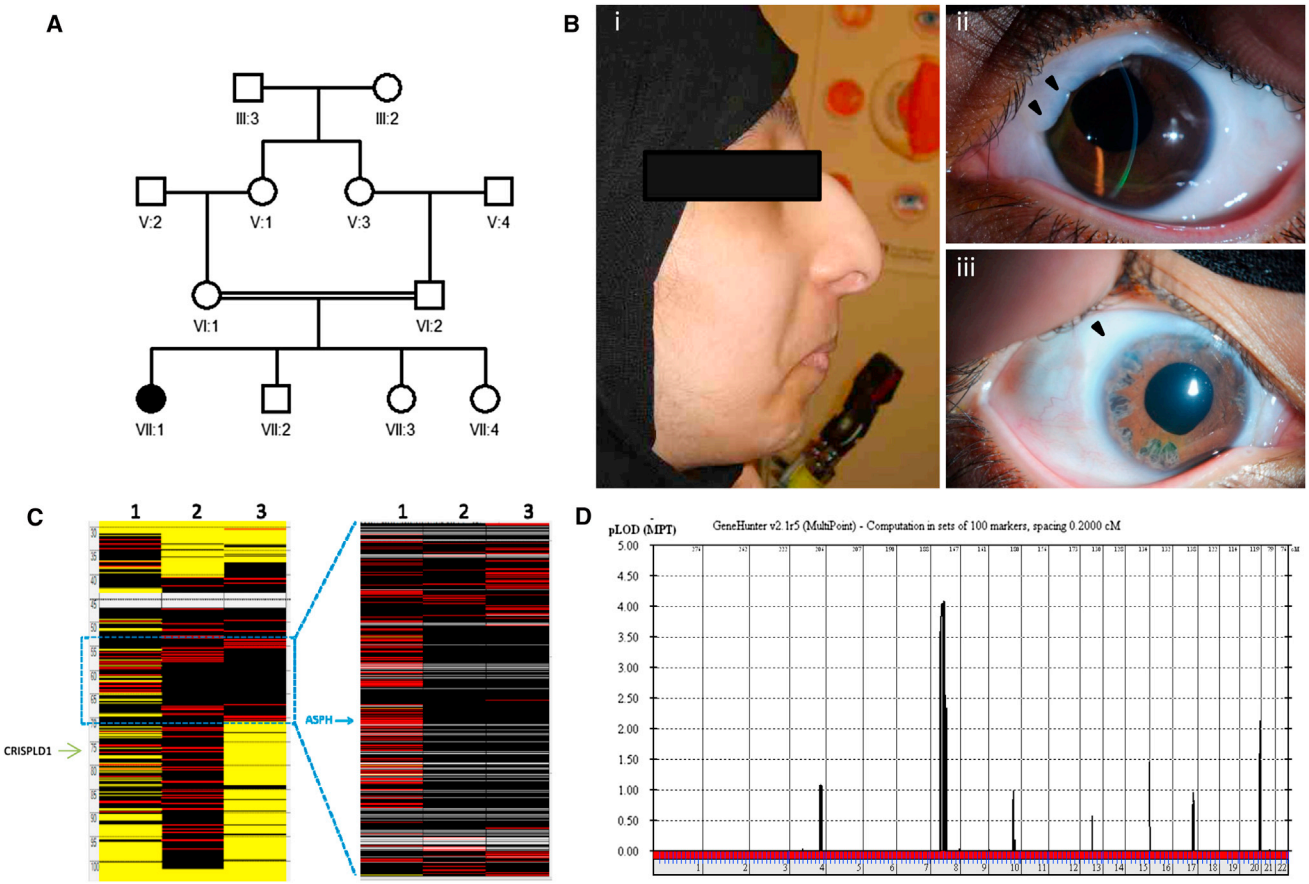

Figure 1. Identification of Individuals with Traboulsi Syndrome and Mapping of Its Associated Locus
(A and B) Pedigree (A) and clinical images (B) of individual 1 from Saudi Arabia. Note the distinct facial features, including a beaked nose, flat cheeks, retrognathia, filtering blebs (black triangles), and patchy iris atrophy. Scleral-fixated intraocular lenses are in place.
(C) Shared autozygous *ASPH* region between the three individuals on chromosome 8. Red denotes differences in calls of homozygous SNPs, revealing the shared haplotype between individuals 2 and 3.
(D) Linkage analysis utilizing the three affected individuals revealed a single significant chromosome 8 peak (LOD > 4) corresponding to the *ASPH* locus.

inherited as part of an autozygous block. Therefore, we enrolled the family in an institutional-review-board-approved protocol with informed consent and proceeded with autozygosity mapping followed by whole-exome sequencing as described before.[6–8] Iterative filtering (based on homozygosity, novelty, predicted pathogenicity, and location within the autozygome) of the resulting 70,436 variants yielded single variants in *ASPH* (MIM 600582; encoding aspartyl/asparaginyl β-hydroxylase [ASPH]) and *CRISPLD1* (Figure S1, available online). We ruled out the variant in *CRISPLD1* (c.614A>G [p .Asn205Ser] [RefSeq accession number NM_031461.5]) because it did not map to the critical locus revealed by the additional affected individuals (see below; Figure 1). The variant in *ASPH*, a 5 bp indel (c.1852_1856delinsGGG [RefSeq NM_004318.3]), is predicted to cause a frameshift with premature truncation (p.Asn618Glyfs*20). However, RT-PCR on blood-derived RNA revealed that this indel results in the complete skipping of exon 22 (r.1765_1900del), in which it resides, and thus causes a frameshift (p.Ser589Glufs*18) that is different from the predicted one, most likely because it removes an exon-splicing element. The predicted result of this frameshift is complete loss of the catalytic carboxyl terminal (Figure 2 and Figure S2). This mutation is absent in 425 in-house Saudi exomes, 100 Saudi controls by Sanger sequencing, and all publically available variant databases. The unaffected siblings were found to be either wild-type or heterozygous for the mutation, whereas the unaffected parents were both heterozygous.

In order to corroborate the human genetics link between *ASPH* and Traboulsi syndrome, we sought additional affected individuals. In this study, we included individual 2, who was the sole individual described by Mansour et al.,[5] and individual 3, corresponding to case II-7 in the pedigree published by Haddad et al.[4] Individual 2 is a Lebanese single female who has lived all her life in an orphanage, so her ancestry is unknown, whereas individual 3 is from a known Druze sect family. Genotyping of these two individuals (2 and 3) revealed a single homozygous interval shared by all three individuals in this study; it corresponds to the *ASPH* locus, which was further confirmed

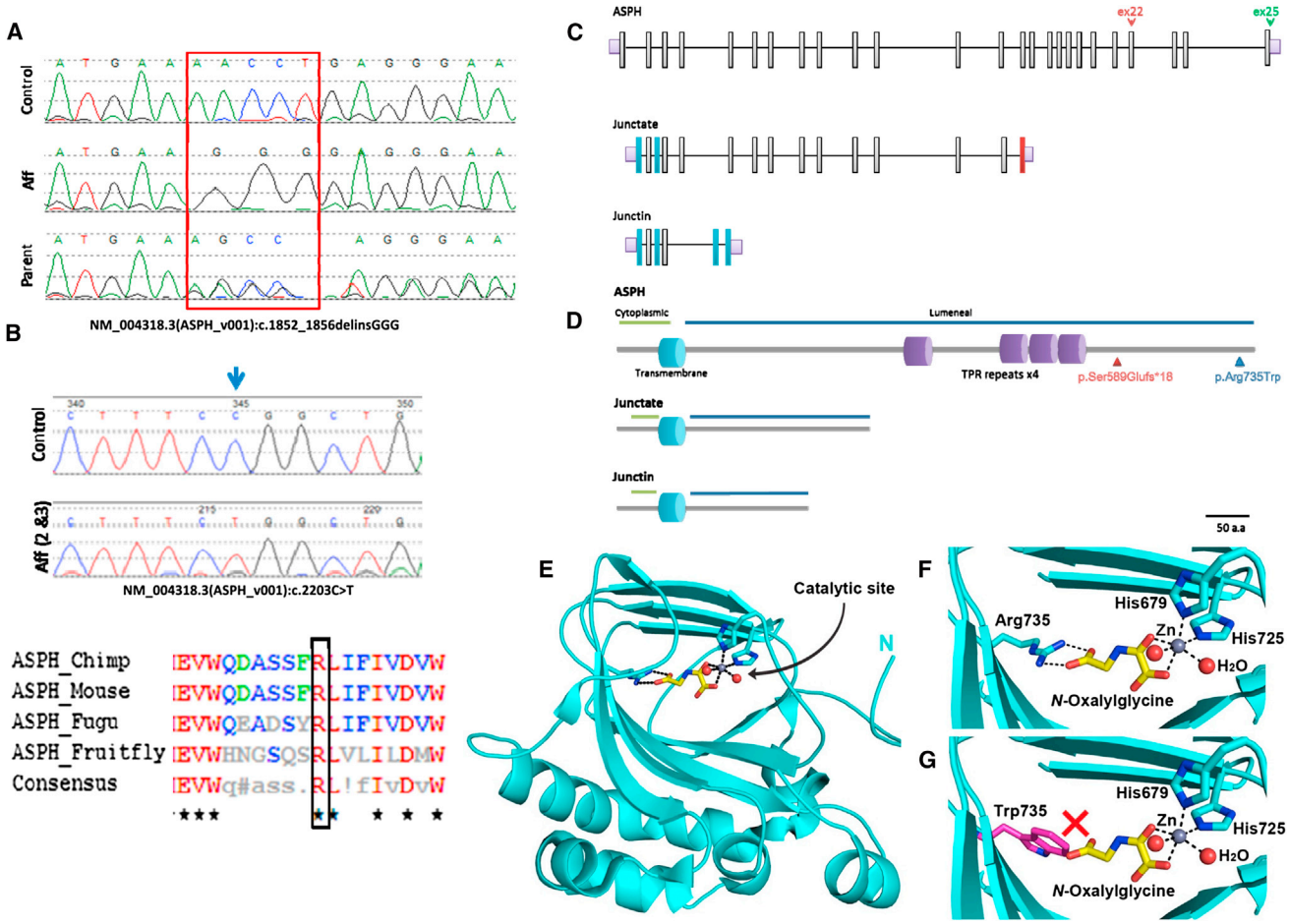

Figure 2. Identification of *ASPH* Mutations that Cause Traboulsi Syndrome
(A) DNA sequence chromatogram of the indel observed in individual 1.
(B) The upper panel shows a DNA sequence chromatogram with the homozygous missense mutation found in individuals 2 and 3. The lower panel reveals strong conversation of the Arg735 residue, which is altered in these two individuals.
(C and D) Schematic showing that mutations found in all three individuals only affect ASPH and not the other two isoforms, Junctin and Junctate.
(E) The crystal structure of human ASPH (residues 562–758) shows the overall structure with the zinc ion and the cofactor analog *N*-oxalylglycine bound at the active site.
(F) A zoomed-in view of the active site shows that the Arg735 side chain forms a pair of salt bridges with a carboxylate group of *N*-oxalylglycine.
(G) A model of the p.Arg735Trp variant in ASPH shows that cofactor binding would be disrupted as a result of the loss of the critical salt bridges and potential clashes with the bulky hydrophobic side chain of Trp.

by linkage analysis (Figure 1). Consistent with the shared haplotype between individuals 2 and 3, sequencing of *ASPH* revealed a shared homozygous missense variant (c.2203C>T [RefSeq NM_004318.3]) that affects an absolutely conserved amino acid residue (p.Arg735Trp) (Figure 2). The frequency of this allele in the NHLBI Exome Sequencing Project Exome Variant Server is sufficiently low (1 in 13,005) to be compatible with being disease causing, even for a disease as rare as FDLAB. Furthermore, we note that this allele is absent in 208 ethnically matched (Lebanese Druze) control chromosomes.

To further support the candidacy of p.Arg735Trp as a pathogenic variant, we set out to study its predicted effect on the protein structure. The crystal structure of the catalytic domain of human ASPH (residues 562–758) has been determined by the Structural Genomic Consortium at University of Oxford (Protein Data Bank ID 3RCQ; NCBI Gene ID 444) (Figure 2). The structure of ASPH exhibits a typical fold that is conserved in all of the Fe^{2+}-α-ketoglutarate-dependent dioxygenases. Eight β strands form a barrel to accommodate the catalytically essential cofactor and metal binding, and additional α helices are present at the amino terminus (Figure 2). The structure includes active-site-bound *N*-oxalylglycine and zinc ion, which mimic the physiological cofactor 2-oxoglutarate (also named α-ketoglutarate) and the catalytic metal ion Fe^{2+}, respectively. It has been established that the binding of the 2-oxoglutarate cofactor enables substrate (side chain of aspartic acid or asparagine) recruitment to the catalytic site and participates in a critical

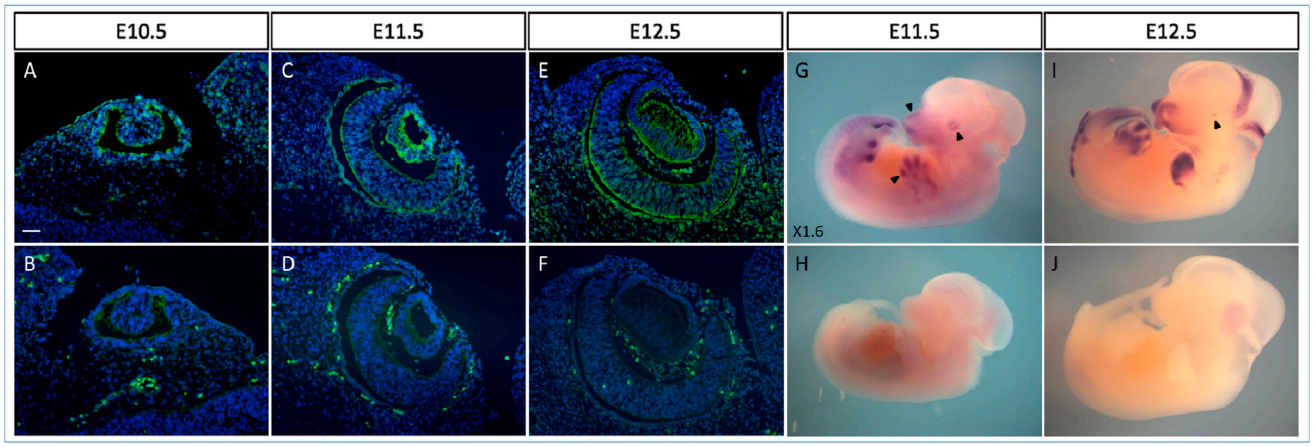

Figure 3. Expression of *Asph* in the Developing Mouse Embryos
(A–F) Immunofluorescent staining of ASPH in E10.5 (A), E11.5 (C), and 12.5 (E) frozen eye sections. Note the strong localization in the developing lens at all three stages. Negative controls are shown in (B), (D), and (F). The scale bar in (A) represents 50 μm.
(G–H) Whole-mount in situ hybridization of *Asph* revealed stronger expression in the limbs, snout, and eye (black triangles) of E11.5 (G) and E12.5 (I) mouse embryos than in those of sense controls (H and J).

step in the catalytic reaction (reviewed in Hewitson et al.[9] and Schofield and Ratcliffe[10]). As revealed in the crystal structure, Arg735 is a critical residue in that it forms a pair of salt-bridge interactions with the cofactor and thus orients it for proper coordination with the catalytic metal ion (Figure 2). The p.Arg735Trp variant would abolish the critical salt bridges and therefore disable the cofactor binding, leading to a loss of ASPH activity (Figure 2). Alteration of a charged Arg to a bulky hydrophobic Trp at position 735 would also potentially interfere with the folding of the protein, further abolishing its cellular function. Thus, this predicted pathogenic variant, together with the variant identified in individual 1, highly suggests that mutations in *ASPH* cause Traboulsi syndrome.

The hydroxylase encoded by ASPH is specific to the aspartic acid and asparagine residues that fall within the consensus motif (CX[DN]4X[FY]XCXC) in EGF-domain-containing proteins.[11] This unusual posttranslational modification, which appears to be EGF specific,[12] was of unclear significance until Dinchuk and colleagues showed that it has developmental consequences.[13] In carefully engineered knockout mice in which only *Asph*, and not the other two isoforms (which code for the two distinct proteins Junctin and Junctate), was disrupted, they showed that ASPH deficiency led to a complete loss of β-hydroxylation of the tested EGF-containing proteins. These mice had a shortened snout that might correspond to the facial malformations of individuals with Traboulsi syndrome. Unfortunately, the eyes were not examined in these mice, so it is not clear whether the mice recapitulated the human eye phenotype. In order to shed more light on the developmental role of *Asph* in the eye and craniofacial region, we set out to examine the developmental profile of *Asph* in developing mouse embryos by whole-mount in situ hybridization. As shown in Figure 3, *Asph* was strongly expressed in the snout, limbs, and eye of embryonic day 11.5 (E11.5) and E12.5 mouse embryos. Immunofluorescent staining of ASPH in E10.5–E12.5 mouse eye sections showed strong localization of the protein in the lens of the developing eye at all three stages (Figure 3). This is highly consistent with a previously published data set that highlights *Asph* as one of the genes in the top two percentiles of genes enriched with lens expression.[14]

We note that the two mutations we identified in this study are specific to the ASPH isoform and are not expected to influence Junctin or Junctate isoforms, which strongly suggests that the pathogenesis of Traboulsi syndrome is restricted to aspartic acid and asparagine hydroxylation activity, given that the latter activity distinguishes ASPH from the other two proteins. Interestingly, virtually all of the genes implicated in syndromic or isolated forms of ectopia lentis (*FBN1* [MIM 134797], *ADAMTSL4* [MIM 610113], *ADAMTS10* [MIM 608990], and *ADAMTS17* [MIM 607511]) encode proteins that harbor EGF domains.[15,16] Thus, it is tempting to speculate that failure of aspartic acid and asparagine hydroxlation of these proteins is a mechanism by which ASPH deficiency results in a lenticular phenotype overlapping that associated with these genes, although other independent disease-causing mechanisms remain possible.

In summary, we provide evidence that *ASPH* is mutated in a distinct form of syndromic ectopia lentis. Our developmental analysis of *Asph* is consistent with previously published phenotypes in *Asph*-knockout mice. Resulting from ASPH deficiency, loss of aspartic acid and asparagine hydroxylation of EGF-domain-containing proteins might affect proteins that have been implicated in the pathogenesis of ectopia lentis, an interesting hypothesis that should be tested by future studies.

Supplemental Data

Supplemental Data include two figures and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2014.04.002.

Acknowledgments

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Web Resources

The URLs for data presented herein are as follows:

NHLBI Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org
RefSeq, http://www.ncbi.nlm.nih.gov/RefSeq
UCSC Genome Browser, http://genome.ucsc.edu/

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