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Supplemental Data

Recurrent Mutations in the Basic Domain of *TWIST2* Cause Ablepharon Macrostomia and Barber-Say Syndromes

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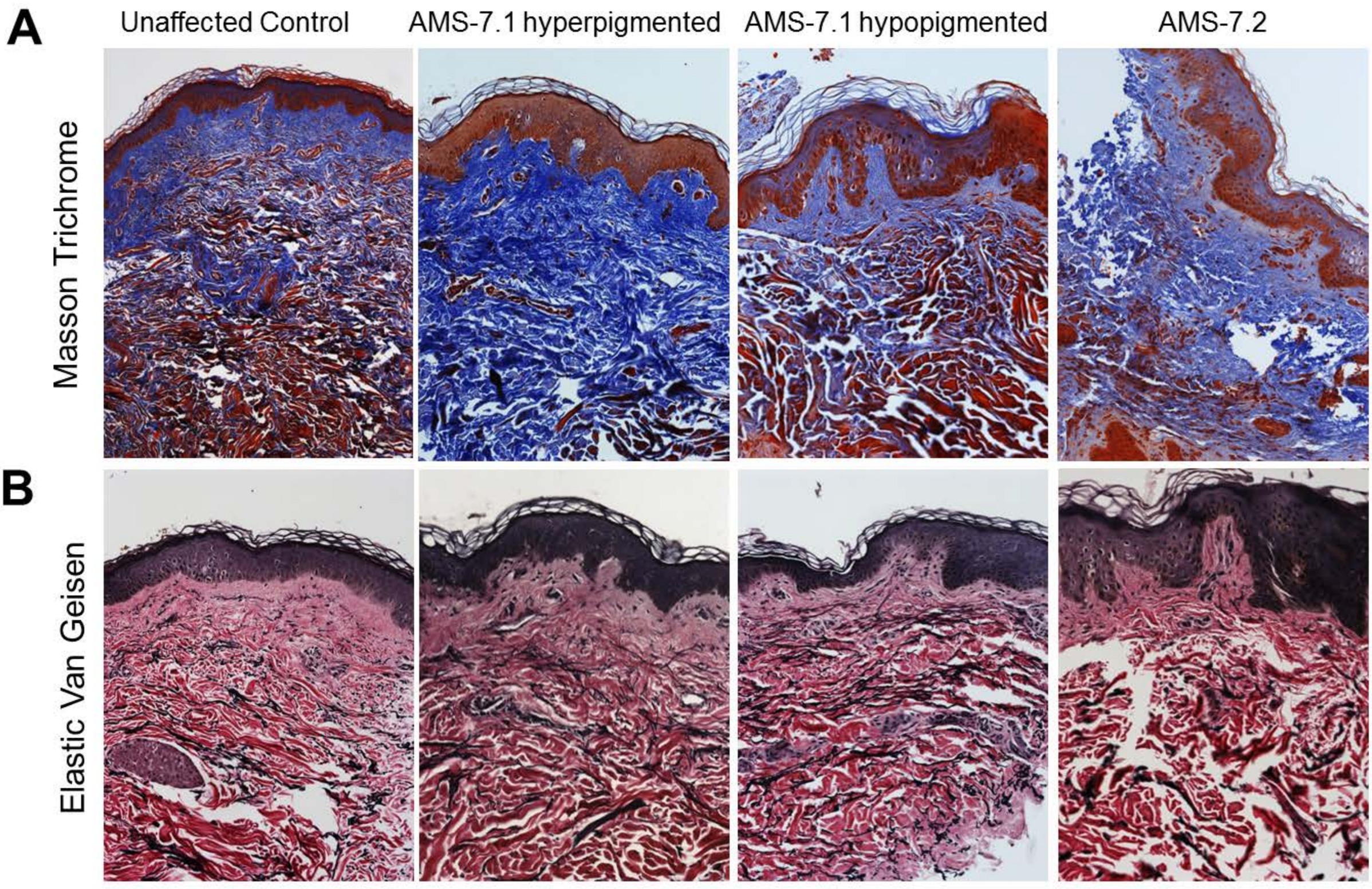
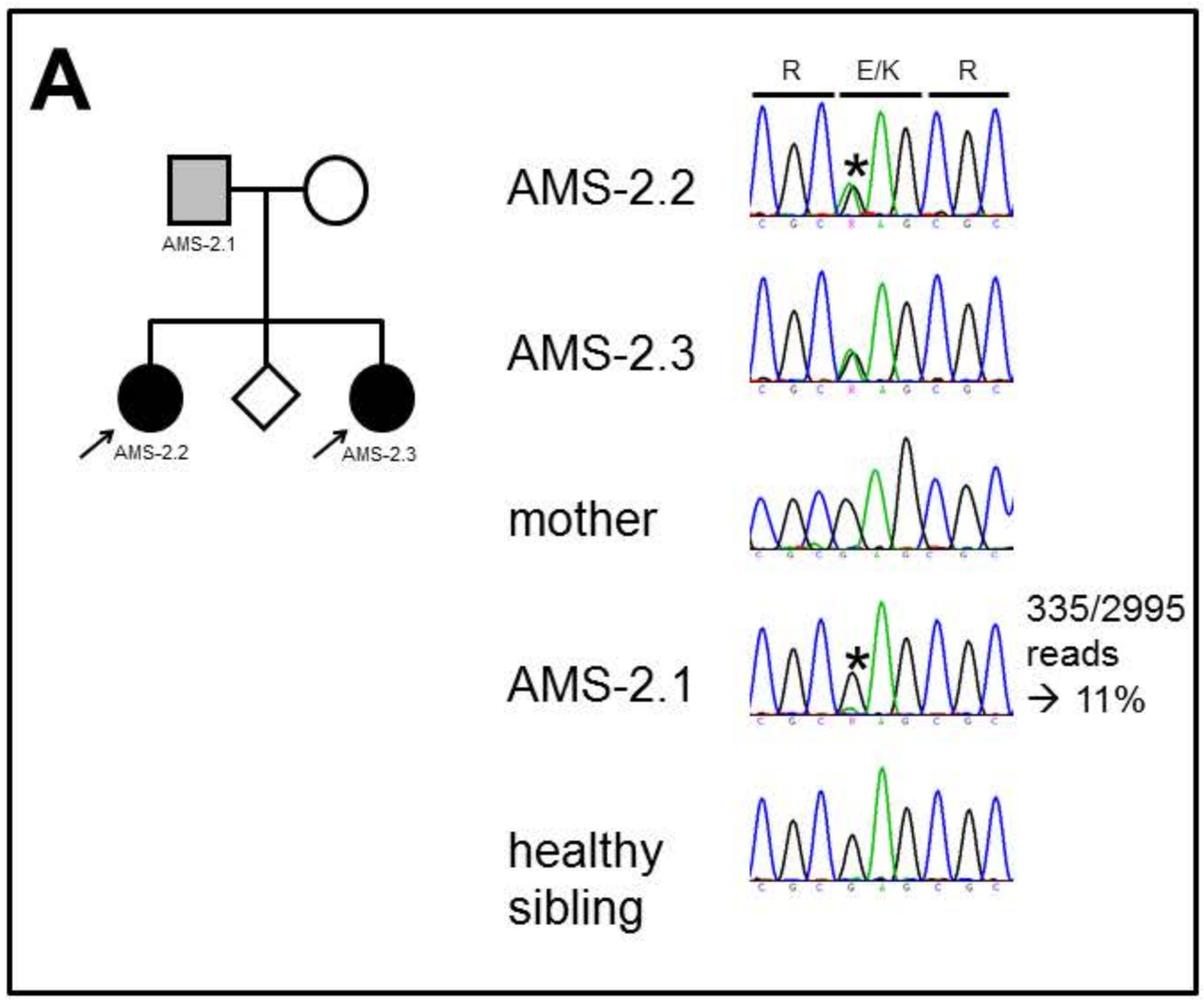
Supplementary Figures

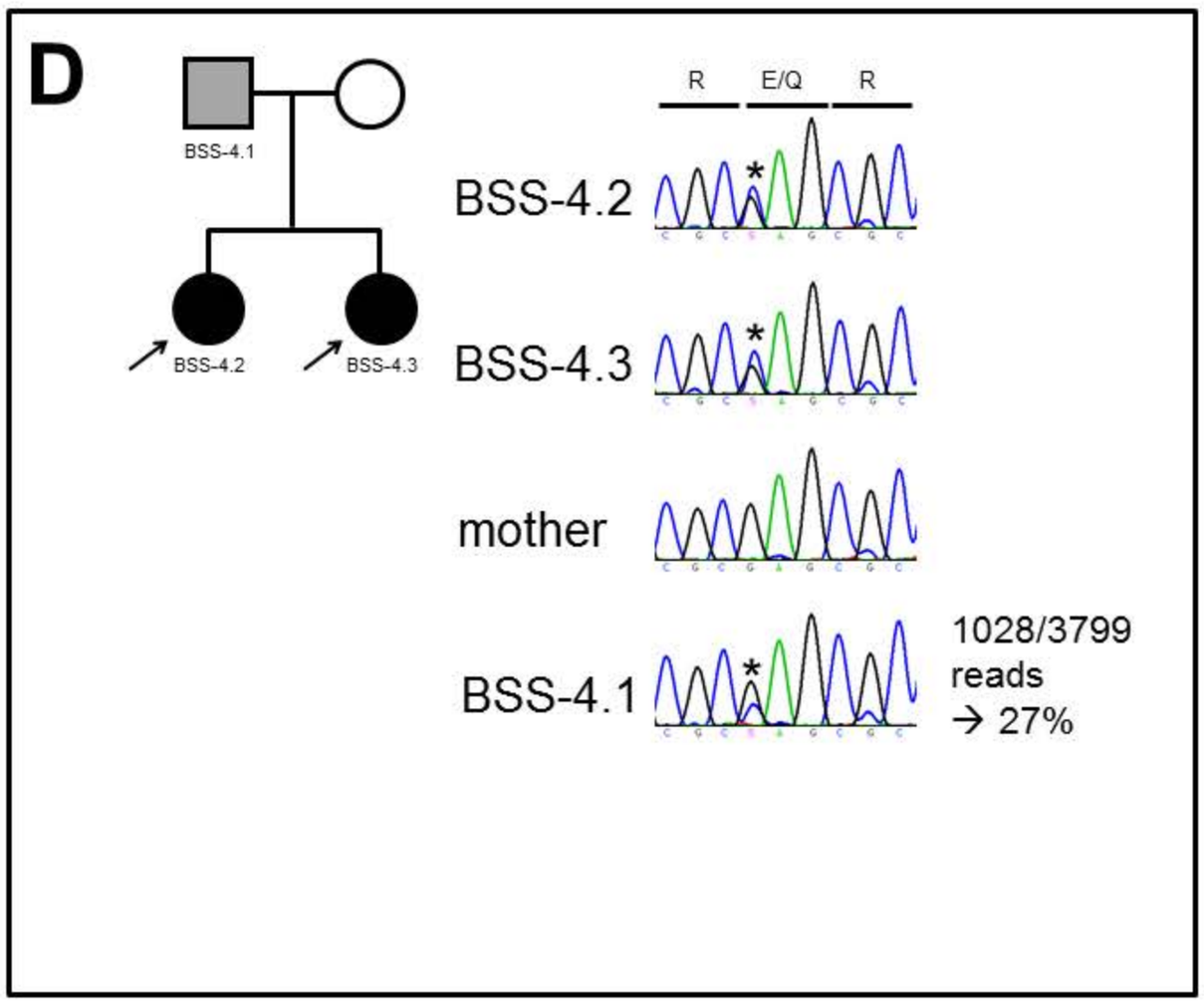
Figure S1

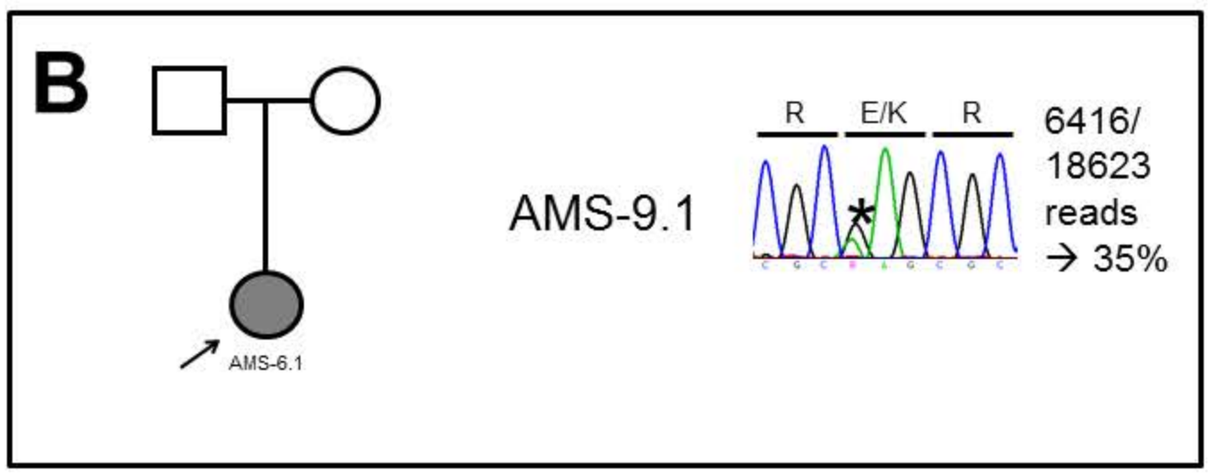


Figures

Figure S2







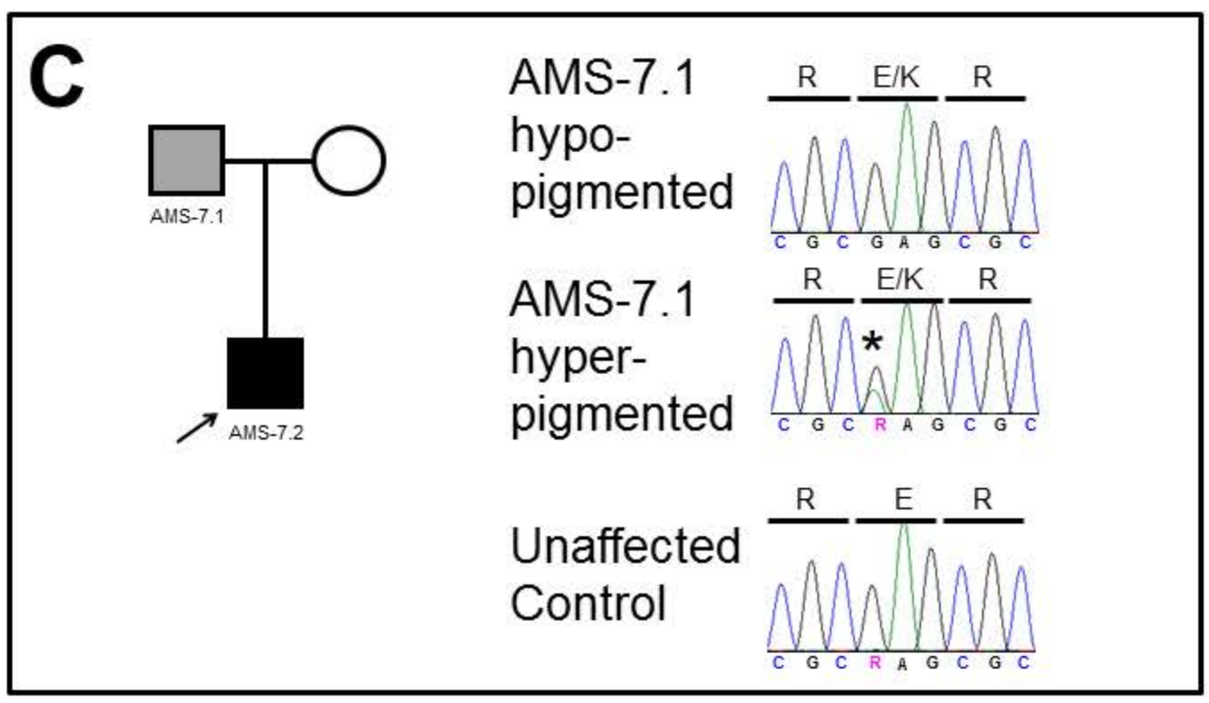
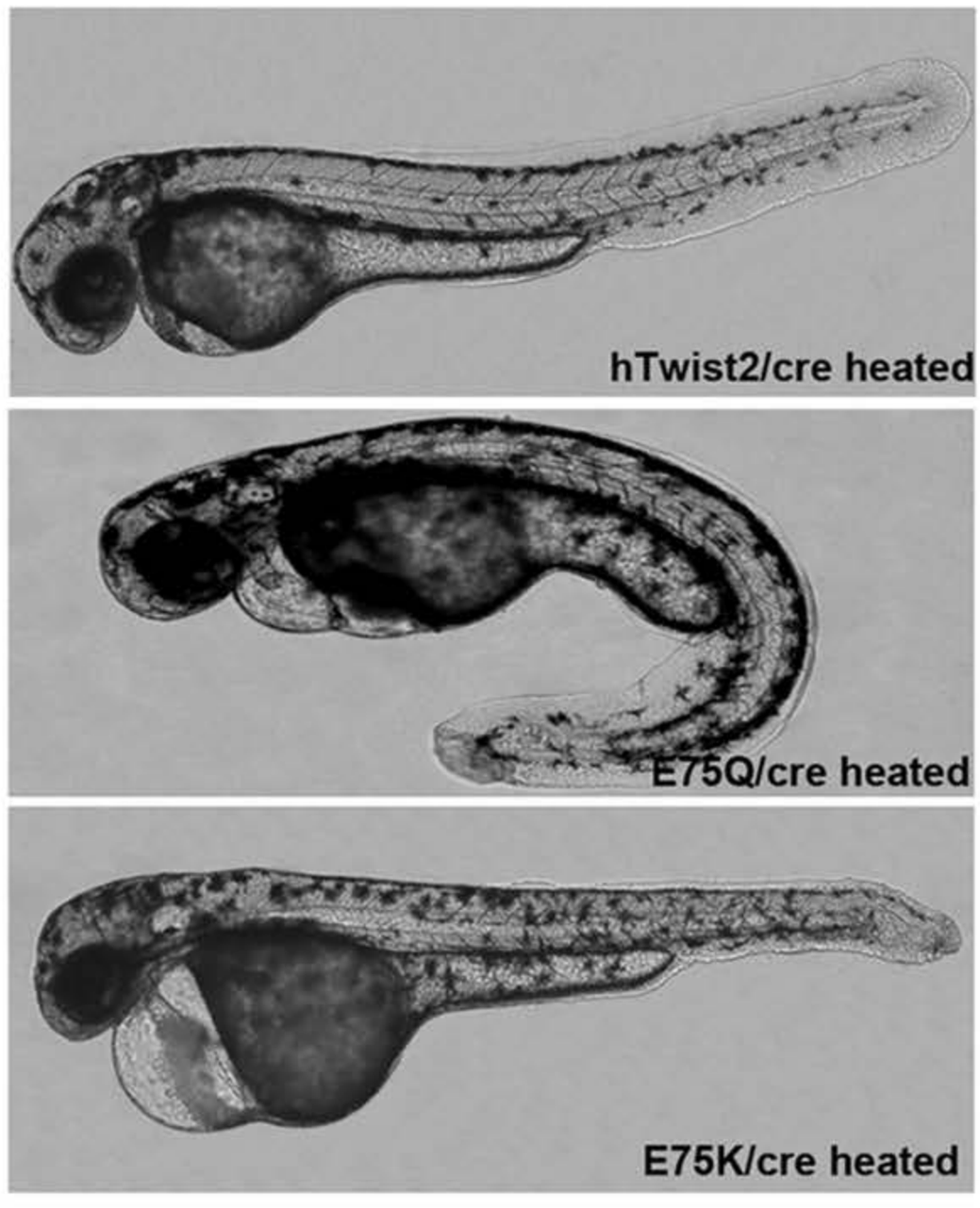


Figure S3


A
hTwist2/cre heated
E75Q/cre heated
E75K/cre heated
B
E75K
(AMS)
E75Q
(BSS)
111
28
23
C
CT956064.3
efemp2b
leprel2
BX296557.7
crabp2b
slc38a2
fgl2
myclb
emilin2a
hmcn2
C8H1orf106
fam107b
hk1
BX548021.2
si:dkey-253d23.4
exoc3l1
ntd5
colec12
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slc38a5a
hhip
syneb
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fbln5
rnf144aa
fmoda
pdgfra
gfra3
E75K
E75Q
-8
-6
-4
-2
0
2
log2 fold change
D
si:ch211-216b21.2
fmoda
pdgfra
efemp2b
gfra3
leprel2
fgl2
E75Q
E75K
-2
-1
0
1
2
log2 fold change

Supplemental Data

Supplemental Figure Legends

Figure S1: Skin biopsies in AMS family 7.

Photomicrographs of full thickness Hematoxylin and Eosin staining of paraffin embedded skin from an unaffected control, AMS-7.1 hyperpigmented (affected) and hypopigmented (unaffected) areas, and AMS-7.2. The tissue was stained with Masson trichrome to demonstrate collagen fibers (blue) or Elastic Tissue Fibers – Verhoeff's Van Gieson (EVG) stains to show elastic fibers (black), which are prominent in the reticular dermis.

Figure S2:Chromatograms showing mosaicism of AMS and BSS families

a, **b**, and **d** were confirmed by next generation sequencing on a Roche GS Junior. **c** results were confirmed by sequencing DNA extracted from dermal fibroblasts derived from hypopigmented (unaffected) and hyperpigmented (affected) skin. Asterisk highlights the mutation.

Figure S3: Zebrafish

a. Defective morphology was observed in all transgenic embryos induced by *Cre* at 2 dpf but mutant p.Glu75Lys (E75K) and p.Glu75Gln (E75Q) embryos displayed stronger phenotype compared to the wild type TWIST2.

b. Venn diagram analysis of differentially-expressed genes in the *hTWIST2* p.Glu75Lys (E75K) and p.Glu75Gln (E75Q) overexpression libraries.

c. Cut-off for selection of genes: padj<0.05. The 28 overlapping genes are listed with the level of up-regulation or down-regulation relative to wild-type *hTWIST2*-overexpressing samples. Data were collected from 3 biologically and technically independent experiments.

d. Seven target genes were chosen from RNA-Seq results for qPCR validation using transgenic lines. Compared to the wild type, p.Glu75Lys (E75K) appeared to be more potent than p.Glu75Gln (E75Q) in suppressing targeted genes. It is worth noting that *fgl2*

was enhanced in p.Glu75Gln (E75Q) but repressed by p.Glu75Lys (E75K), suggesting some difference between the two mutations in inducing the disease.

Supplemental Table

TABLE S1. Primers used for sequencing cloning, and zebrafish experiments

Primer Name	Primer Sequence
TWIST2_FLAG_HA_GC_F1	CACCATGGACTACAAGGACGACGATGACAAAGTCAAG TCTACCCATACGATGTTCCAGATTACGCTGCTGCTATG GAGGAGGGCTCCAGC
FLAG_HA_mutagenesisfix_F	GATGACAAAGTCAAGCTCTACCCATACGATG
FLAG_HA_mutagenesisfix_R	CATCGTATGGGTAGAGCTTGACTTTGTCATC
TWIST2GATERSTOP	CTAGTGGGAGGCGGACAT
TWIST2SangerSequencingF	CAGAGCCTTTCCAGCAACTC
TWIST2SangerSequencingR	AGCGTGGGGATGATCTTG
TWIST2_E75Q_mutF	GCGCTGGCGCTGGCGCACGTTGG
TWIST2_E75Q_mutR	CCAACGTGCGCCAGCGCCAGCGC
TWIST2_E75A_mutF	TGCGCTGGCGCGCGCGCACGTTG
TWIST2_E75A_mutR	CAACGTGCGCGCGCGCCAGCGCA
TWIST2_R77_Q78dup_mutF	CGTGCGCGAGCGCCAGCGCCAGCGCACCCAGTCGCTC
TWIST2_R77_Q78dup_mutR	GAGCGACTGGGTGCGCTGGCGCTGGCGCTCGCGCACG
TWIST2_E75K_mutF	CAACGTGCGCAAGCGCCAGCG
TWIST2_E75K_mutR	CGCTGGCGCTTGCGCACGTTG
fg12_F	TGGTCAATAAAATCAGCAGCAC
fg12_R	CCTCTGAGTCTTCCAGCTCAAT
efemp2_F	GGTGGTTACCTGTGTCTTCCTC
efemp2_R	CCTGGTTCACACTCACAAGAGA
leprel2_F	CAGGCAGTGGATTATCATCAGA
leprel2_R	AGACACTTCGACTCATGCAGAA
gfra3_F	CTGTTTAGAGGCTCTCCAGGAA
gfra3_R	AAACTATGGAGGCCAGTTTTGA
pdgfra_F	GACGTTCCTGAGGTTGTAGACC
pdgfra_R	AATGAGCTCTCGTGAACTGTGA
fmoda_F	GGCCAATCAGATCAAAGAGTTC
fmoda_R	GATAAAACAAATTGGCTGCACA
si:ch211-216b21.2_F	CAGATAAGCGCATGTTTCTGTC
si:ch211-216b21.2_R	GGAGCTTCTCGAGTGACAAAGT

Supplemental Methods

Histology

Skin biopsy specimens were fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin. After deparaffinization of 4-5 μm sections, the slides were subjected to a series of rehydration steps. Slides were stained with Masson Trichrome and Elastic Tissue Fibers - Verhoeff's Van Gieson (EVG) stains following standard protocols.

Zebrafish

Stable transgenic zebrafish lines expressing human wild type and mutant human *TWIST2* (p.Glu75Lys and p.Glus75Gln) under the control of a Cre-loxP inducible system were generated using a Tol2 based transgenic technology. Wild type or mutant *TWIST2* cDNA were placed downstream of zebrafish β-actin promoter, in which mCherry-STOP signal with two loxP elements at the both ends was placed between *TWIST2* gene sequence and β-actin promoter. Upon introduction of Cre, the mCherry-STOP cassette will be removed, functioning as an inducible element. Phenotypes of transgenic embryos were analyzed upon induction of Cre by heat at 38.5˚C for 30 minutes at 24hpf using heat-shock hs-Cre (+/-) and Twist2 (+/-) double transgenic zebrafish embryos. For qPCR, 60 transgenic embryos were heated at 38.5˚C for 30 minutes at 80% epiboly stage and then collected for RNA preparation in Trizol three hours later.