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Supplemental Data

Loss-of-Function Mutations in *HOXC13*

Cause Pure Hair and Nail Ectodermal Dysplasia

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Figure S1. Multiple Alignment of the Region Containing Substitution Site Asn470 of GLI1 in Different Mammalian Species

Note that Asn470 is not conserved in Tasmanian devil (black highlighted).

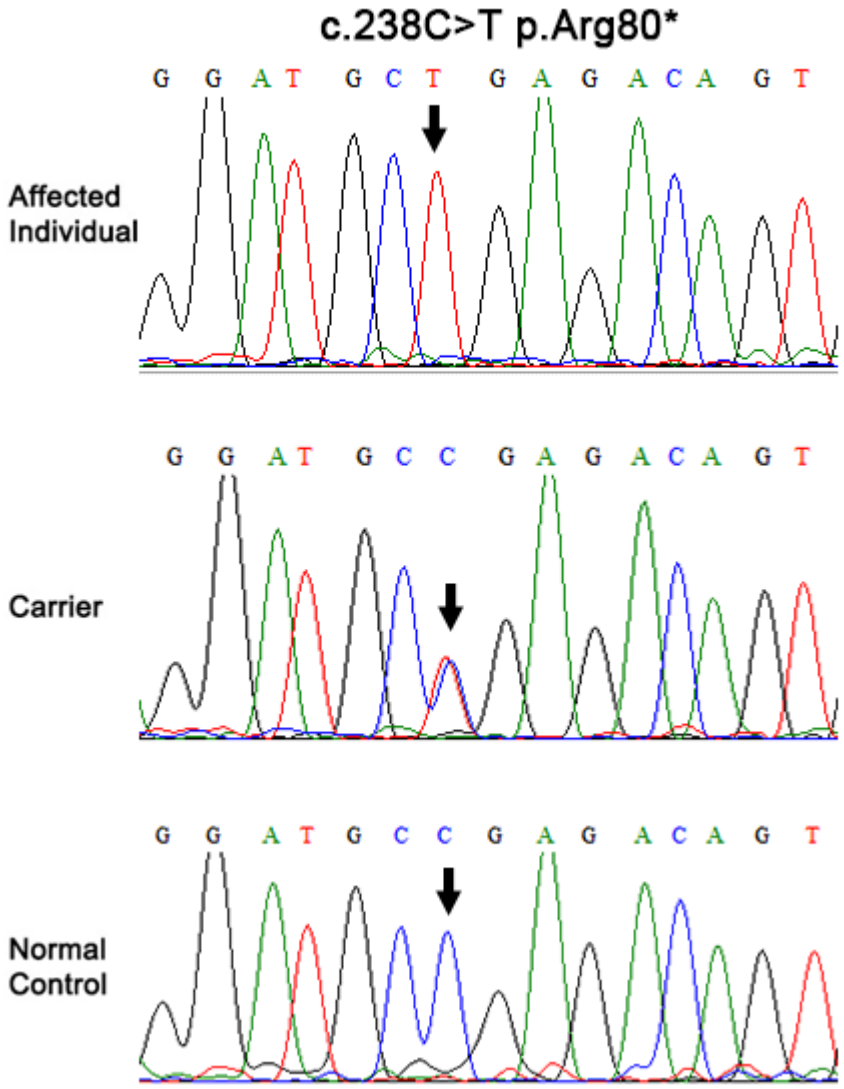

Figure S2. The Nonsense Mutation in the *AMHR2* Gene in the Chinese Family Causing Persistent Müllerian Duct Syndrome

Sequence chromatograms show the c.238C>A mutation (p.Arg80*) in individual V2 (top panel) in a homozygous state and in his father in a heterozygous state (middle panel). This homozygous mutation is also found in individuals IV10 and V3, the latter of whom has no symptoms of persistent Müllerian duct syndrome.

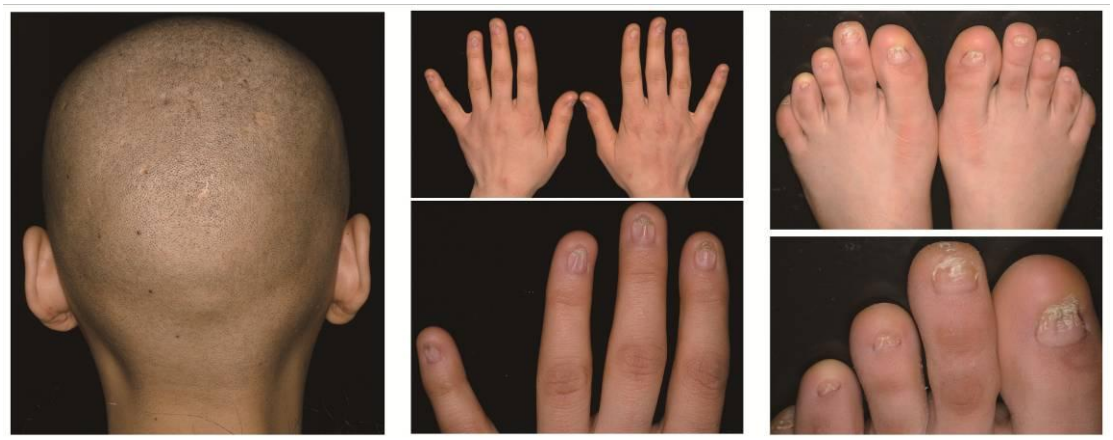

Figure S3. An Afghan female with hypotrichosis (left) and nail dystrophy (middle and right)

Table S1. Summary of Exome Sequencing and the Filtration Procedure

Individual	**IV5**	**IV6**	**V2**	**V3**
Raw data yield (Mb)	4077	4307	4566	3687
Data mapped to target region (Mb)	4444.01	4701.69	5121.41	3724.07
Mean depth of target region	100.47	106.54	115.78	84.39
Coverage of target region (%)	99.21	99.31	99.28	99.26
Fraction of target covered >=10X	96.42	96.76	96.57	96.04
Capture specificity (%)	75.16	75.41	76.59	69.35
Read coverage of CCDS[a] exons	264782	264096	264850	264268
Read coverage of CCDS genes	18378	18334	18375	18343
Total number of variants	104552	104517	102337	98900
Variants in exons	26391	26289	26010	25676

SNVs data filtration	**Filtration methods**	**Variants number for V2**	**Variants number for V3**
Step 1	Homozygous variants that is heterozygous in individual IV5 and IV6 and not in the 1000 Genomes Project, the dbSNP134, HapMap databases, YH database, BGI inner databases.	45	35
Step 2	Step 1 data in both individual V2 and V3	8	
Step 3	Step 2 data predicted not tolerated by SIFT software	5	
Step 4	Step 3 data expressed in skin	3	
Step 5	Step 4 data excluded from 200 normal controls	2 (*HOXC13* and *GLI1*)	

[a]Consensus coding sequence

Table S2. Primer Pairs for *HOXC13* Sequencing

	Forward Primers (5'-3')	**Reverse Primers (5'-3')**	**Annealing Temperature (°C)**	**Amplicon Size (bp)**
Exon-1	GAAACAGGAGCGAGGTGTCT	AACGTGTCGGAGCAGATTTC	62	1000
Exon-2	AGCCTCGGGTCCTCTATCTC	CTTTCCGTGGGTTCGGTTAT	61	394

Table S3. Primers Used in Deletion Analysis

Amplicon	Position	Forward Primer (5'-3')	Reverse Primer (5'-3')	Size (bp)
1	chr12:54300777-54301505	GGAGCAGAACAGAAACAGTGGAC	CAGGTTCCGTCTGTCTGGTCTT	728
2	chr12:54306337-54306799	CAGGAGGAGAAGTTTTGGTGGT	CATTATTGCACTCCAGCTTAGGC	462
3	chr12:54307568-54308146	GCTGGCTGTGTAACTCTCTTCAT	TAAGAGATAATCCGCCAACAGC	578
4	chr12:54308194-54308812	TTGAGCCCAGGCATAATTTC	AAATAGCACCAAGAGCAAAACC	618
5	chr12:54309789-54310865	GCAGCCGTCACACTATCCAC	GCAGAGTGAAAAACCCATCAAG	1076
6	chr12:54317881-54318514	GACCCTGCTACTGAATCCGAAC	GTGGCACCAGCACTCTCTCAG	633
7	chr12:54323589-54324506	ATGTTTGACGGGGAGAGGGTT	GGCATAGTCACACACGCAATCC	917
8	chr12:54327928-54328645	CAAGTTTGGGTTTAAGAGCGG	ACAAAGAGCCTCACCACCATG	717
9	chr12:54332362-54332760	ATCAGCACAGACTTTTTCCCTTTCGC	CCGCGCTGTCCTCATAGACGTACAT	398
10	chr12:54332369-54332874	CAGACTTTTTCCCTTTCGCCC	TGCCCAGACCATCCATGCT	505
11	chr12:54332528-54333611	TGCGTAGAGGGAATGTAGGG	CGTGTCGGAGCAGATTTCTT	1083
12	chr12:54333601-54333900	GCTCCGACACGTTCTCTGTAGCTGC	TGTCTAGTGCCTGAACGATCCTGCG	299
13	chr12:54334578-54334857	AGCCACAGATGTCTCCCGACAGTGC	TCAGAAGTCAGGGTGCCTAGTGTAGG	279
14	chr12:54335518-54335815	CAGCAGCTATGTGCTCCCACAATCCC	ATTGCCTCCATCTCCCACACCAGCC	297
15	chr12:54336590-54337360	CTAAATCAATCCTTGGCATCCT	GCCTCATTTGACTGTCTTCTGG	770
16	chr12:54337449-54338176	GTCCATGAGAGAAAGCAGACCC	AGATGAGTCTTCACGCCTTTGG	727
17	chr12:54338726-54339233	CTCTATCTCAGTCCAGCCGCTTGCC	AGGCTGGGGATGGGATAGGGAGTTG	507
18	chr12:54339786-54340159	AGGACAAAGACTCCAGCCACGCTGC	ACCAGTGGGAAGGGAAGAGGCAAGG	373
C	chr8:42301935-42302488	TTTAAGCACATATTCGCCAGA	CTTCCAGTTACTCATGGCAAC	553

Table S4. GLI1 (NM_005269.2) Primers for PCR Amplification and Sequencing

Amplicon	Position	Forward Primer(5'-3')	Reverse Primer(5'-3')	Size
Ex2-3	chr12:57,857,293-57,858,010	CGGCTCAGTGATGCTCTTTTC	ACTTCTTGATGGTGCTCTGACG	718
Ex4-5	chr12:57,858,331-57,859,186	AGATAGCATCAATAGGGCAAAG	AAGGAGTGAACCCCAGCA	856
Ex6-8	chr12:57,859,342-57,860,316	TTCTGCTTACTTCCACCCTCAT	GCCAACACAGTCACACAAACAT	975
Ex9	chr12:57,860,948-57,861,379	AAGTTCTTGCCTGCCCTTGTC	CTCTGTGGAGTGGGTGCTGG	432
Ex10	chr12:57,861,696-57,862,158	GTTGTCACCACTTTTTCCCATT	GCCCAAAGAGCAACACTAAGAC	463
Ex11	chr12:57,863,060-57,863,562	AGTTTGCTCCCCCATCTCTTAC	CCTCAAGGGTGACTTCCTCCT	503
Ex12-1	chr12:57,864,060-57,864,873	GCTCCTTGACCATCCTACCTT	GGAACTCACCCCATGTTTCTT	814
Ex12-2	chr12:57,864,362-57,865,262	TGGTCTTCCCATGCCTCCTT	CCCACCCTCCCACAGTAGCT	901
Ex12-3	chr12:57,864,913-57,865,963	GTGGAACCTACAGCCAGTGTCC	AGCCCATACCTCCCATCCCT	1051

Table S5. Primer Pairs for qRT-PCR

	Forward Primers (5'-3')	Reverse Primers (5'-3')
18S rRNA	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
ACTB	CTGGAACGGTGAAGGTGACA	AAGGGACTTCCTGTAACAATGCA
HOXC13	GGCTAGCAAGTTCATCACCA	AGATGAGGCGCTTTCGATTT
FOXN1	CAAGAGAGAAAAGCTGGGCT	GTGTGCCCCATAAGTAGGTC
DSG4	AAATGATGGGAATTGGTTCG	TGGATGAAATGCAGGTCCTT
KRT85	AAAGAAGGACGTGGACTGTG	CAGGAAGCTAGACTCCTCCA
KRT35	CTGACCCTGTGCAAGTCTGA	CTCGGTTCAGGTCAACAGGT