Supplementary materials

Chr.	SNP 1	SNP 2	Position 1	Position 2	Size (bp)
5	rs341893	rs2578628	8,467,900	10,242,162	1,774,262
5	rs247206	rs2190622	128,085,409	135,414,280	7,328,871
10	rs993175	rs7085249	4,450,357	5,243,017	792,660
10	rs562950	rs4554798	131,032,716	134,149,946	3,117,230
11	rs12790085	rs7939047	131,301,616	133,856,832	2,555,216
18	rs9951431	rs17090062	27,368,785	53,763,024	26,394,239
19	rs11085177	rs4239642	6,520,490	14,779,550	8,259,060

Table S1. Linkage analysis results showing heterozygous genomic loci shared between all cases in the studied family.

Chr.	Position	Reference Allele	Sample Allele	Variation	Gene Region	Gene Symbol	Transcript Variant	Protein Variant
18	28,979,241	G	A	SNV	Exonic	DSG4	c.1012G>A	p.D338N
18	33,795,501	A	G	SNV	Exonic	MOCOS	c.1358A>G	p.N453S
19	10,449,565	C	T	SNV	Exonic	ICAM3	c.136G>A	p.G46R
19	11,170,525	G	A	SNV	Exonic	SMARCA4	c.4828G>A	p.E1610K
19	11,558,353	GAG		Deletion	Exonic	PRKCSH	c.966_968delGGA	p.E325del

Table S2. Whole-exome sequencing results following variant analysis showing variants common to both tested cases.

Gene Symbol	Comments
DSG4	- Protein expression profile does not match the phenotype: highest expression in bone marrow stem cells, heart, and hair follicles. - Although the specific amino acid is relatively conserved throughout evolution, several lower organisms carry the specific D>N substitution. - Homozygous mutations in this gene were associated with hypotrichosis, an unrelated phenotype. - According to gnomAD this gene has a very low pLI (probability of loss-of-function intolerance).
MOCOS	- According to gnomAD the allele frequency of this variant is 21/251060; pLI for this gene is very low. - This gene is mostly expressed in tissues unrelated to the phenotype: mononuclear cells, lung, adrenal, liver, placenta, testis. - This particular amino acid is not conserved at all throughout evolution; many organisms carry a p.N453D substitution. - Homozygous mutations in this gene are associated with Xanthinuria, an unrelated phenotype.
ICAM3	- This gene is mostly expressed in tissues unrelated to the phenotype: blood and immune cells, mucous membranes. - The altered amino acid is relatively conserved throughout evolution; however, 3 different substitutions exist at this position in 3 different organisms; moreover, this amino acid is missing in ICAM3 orthologs in many lower organisms. - According to gnomAD this gene has a very low pLI.

PRKCSH	- According to gnomAD the allele frequency of this variant is 75/244564, with no homozygotes; pLI for this gene is very low. - The deleted amino acid is not conserved throughout evolution at all; present only in 2 high primates and humans; there is an E>V substitution in chimps. - Variants in this gene were previously associated with autosomal dominant polycystic kidney disease, an unrelated phenotype.

Table S3. Detailed description of variant filtration process for all otosclerosis-linked variants identified.

Gene/oligo	**primer**	**Sequence**
SMARCA **- Human**	Forward	5'-CTGCCCAGATCTATGAAGAC-3'
	Reverse	5'-GGGAGGGCTGTGAAAAGCTG-3'
Smarca4 **- Mouse**	Forward	5'-GTCCTTGCAGATCTATGAGG-3'
	Reverse	5'-AGCAAACCGTTAAGAGAGGC-3'
sgRNA		TGAAGGCGAGGAAAGCGAGG
ssODN*		GCTGCAGCGTCTGCCCTTCCCCCTTACTCACACTCA GACTCGGAGCCTTCCTCCTCGCCCTCCTCCTtCTCCT CGCTTTCCTCGCCTTCACTGTCGTCCTCCTTCTCAAT CTTCTGCCGTACGCTGGTGAAGA

Table S4. Oligos used to generate knock-in mice and primers used for Sanger sequencing. *Mutation site denoted with a lower-case letter. ssODN, single-stranded donor oligonucleotides; sgRNA, short-guide RNA.