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Short communication

# Identification of the first multi-exonic *WDR72* deletion in isolated amelogenesis imperfecta, and generation of a *WDR72*-specific copy number screening tool

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ABSTRACT

Amelogenesis imperfecta (AI) is a clinically and genetically heterogeneous disorder of tooth development which is due to aberrant deposition or composition of enamel. Both syndromic and isolated forms exist; they may be inherited in an X-linked, autosomal recessive, or autosomal dominant manner. *WDR72* is one of ten currently known genes for recessive isolated AI; nine *WDR72* mutations affecting single nucleotides have been described to date. Based on whole exome sequencing in a large consanguineous AI pedigree, we obtained evidence for presence of a multi-exonic *WDR72* deletion. A home-made multiplex ligation-dependent probe amplification assay was used to confirm the aberration, to narrow its extent, and to identify heterozygous carriers. Our study extends the mutational spectrum for *WDR72* to include large deletions, and supports a relevance of the previously proposed loss-of-function mechanism. It also introduces an easy-to-use and highly sensitive tool for detecting *WDR72* copy number alterations.



## 1. Introduction

Amelogenesis imperfecta (AI) denotes a group of inherited dental disorders in which an abnormal formation of enamel results in discoloration, sensitivity and fragility of teeth. It can occur in conjunction with changes in other intra- or extra-oral tissues (syndromic forms), or without any comorbidities (isolated forms). A phenotype-based classification scheme recognizes hypoplastic, hypocalcified and hypomaturation AI, with the latter two sometimes collectively referred to as hypomineralized AI. Clinical distinction between these entities, however, becomes difficult once post-eruptive changes have occurred (Witkop, 1988). As in many other genetically heterogeneous disorders, a genetic classification scheme is currently taking over (Gadhia et al., 2012).

Isolated AI may be inherited in an X-linked, an autosomal dominant, or an autosomal-recessive manner. With at least ten causative genes, the recessive forms appear to be genetically most diverse (Poulter et al., 2014). One of these genes is *WDR72*, bi-allelic mutations of which cause isolated hypomaturation AI (El-Sayed et al., 2009). Based on targeted resequencing, a total of nine pathogenic *WDR72* variants have been identified in patients to date (El-Sayed et al., 2009; Lee et al., 2010; Wright et al., 2011; El-Sayed et al., 2011; Kuechler et al., 2012; Katsura et al., 2014; Prased et al., 2016). As all but one are either non-sense or frameshift alterations, a loss-of-function mechanism has been suggested (El-Sayed et al., 2011). Copy number mutations, which very likely would also lead to loss of protein function, have not yet been found in *WDR72*.

Here we report the first association of a multi-exonic *WDR72* deletion with isolated AI in a large consanguineous family. We also introduce and validate a genetic screening tool for easy detection of *WDR72* copy number alterations in routine and diagnostic settings.

*Abbreviations:* AI, amelogenesis imperfect; DNA, desoxyribonucleic acid; MLPA, multiplex ligation-dependent probe amplification; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; UCSC, University of California Santa Cruz; *WDR72*, WD REPEAT-CONTAINING PROTEIN 72.

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## 2. Patients and methods

### 2.1. Patients and controls

The patients were recruited in an outpatient clinic, and referred for expert clinical and diagnostic workup to the Department of Preventive and Paediatric Dentistry at Jena University Hospital. After informed consent was obtained, genomic DNA was extracted from whole blood by an in-house salting-out procedure. Twelve anonymised genomic DNAs from unrelated individuals served to test the general performance of a newly developed multiplex ligation-dependent probe amplification (MLPA) probe set. The study was approved by the local ethics committee.

### 2.2. Sequencing

DNA samples from patients VI-3 and VI-4 (see Fig. 1A) underwent exome sequencing by combining the TruSeqExome enrichment Kit and a HiSeq2000 platform with 2 × 100-base pair paired end reads (Illumina, San Diego, CA). Reads in FastQ files were aligned to the hg19 reference sequence with the Novoalign software (Novocraft, Petaling Jaya, Malaysia). Read counts per exon were calculated using BedTools (Quinlan and Hall, 2010).

### 2.3. Multiplex ligation-dependent probe amplification

The *WDR72* genomic sequence (NM_182758.3) was downloaded from the UCSC genome browser (www.genome.ucsc.edu) with exons, repeats and common SNPs highlighted. MLPA probes were developed against 14 of the 20 exons. The ligation site for the exon 10 probe overlapped with nucleotide c.997A to enable screening for variant c.997A>T (Kuechler et al., 2012) during validation of the probe set. Probe design followed recommendations provided at www.mlpa.com. Four probes from previously established probe sets served as reference. Oligonucleotide sequences for all probes are available upon request. MLPA reactions utilized reagents from MRC-Holland (Amsterdam, The Netherlands), and were performed according to the manufacturer's recommendations. Data analysis was done as described previously (Beetz et al., 2006).

## 3. Results

### 3.1. Numerous patients in several branches of a highly consanguineous family suffer from amelogenesis imperfecta

The female index case and its two affected siblings are of Turkish origin; their parents are third degree cousins (Fig. 1A). All three patients had been diagnosed with clinically isolated hypomineralized AI. Both primary and permanent teeth had rough, soft enamel which lacked translucency and showed a yellowish-brownish colour (Fig. 1B; Supplementary Fig. 1). There was evidence for enamel loss due to chipping and attrition. Defects were most pronounced at the occlusal surfaces of the lateral teeth. Phenotypic variability was low except that the primary teeth of male patient VI-5 seemed less severely affected. Sensitivity to thermal stimuli was reported by all patients for all teeth. Radiographic investigations revealed a thin enamel layer which can hardly be differentiated from the dentin layer (Fig. 1C; Supplementary Fig. 1). Two grandnephews and one grandniece of the father (VII-1, VII-2, VII-3) as well as three paternal cousins of the mother (V-12, V-13, V-14) were reported to have similar symptoms; the latter were also born to consanguineous parents (Fig. 1A).

### 3.2. Coverage gap in whole exome sequencing, and failure of targeted PCR amplifications suggest homozygous deletion of exons 13 to 18 of WDR72

Given the genetic heterogeneity of autosomal recessive AI, the large size of the known genes, and the strong evidence for as yet unrecognized genetic forms we decided to analyze patient DNA by whole exome sequencing. The initial step of our envisaged strategy consisted of filtering for known AI genes. When compiling the corresponding data, we noticed absence of sequence reads for exons 13 to 18 of *WDR72*. As one explanation for this coverage gap was the presence of a multi-exonic deletion, we targeted the critical exons by PCR. Failure to amplify exons 13 and 18, but not exons 12 and 19 from patient

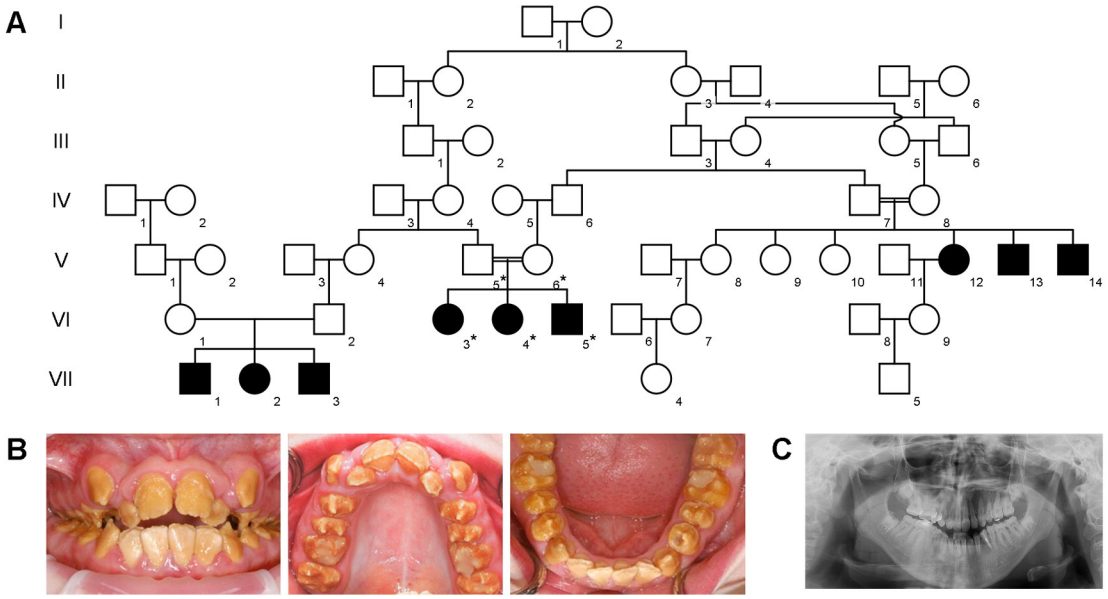


**Fig. 1.** Familial amelogenesis imperfecta. (A) Pedigree. Note the presence of multiple family branches with consanguinity. Stars denote individuals which were available for clinical and genetic investigations. (B) Dentition of patient VI-3 (see Supplementary Fig. 1 for corresponding images of the other patients). (C) Radiograph of patient VI-3 (see Supplementary Fig. 1 for corresponding images of the other patients).