



Pollitt syndrome patients carry mutation in *TTDN1*



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ABSTRACT

Complete human genome sequencing was used to identify the causative mutation in a family with Pollitt syndrome (MIM #275550), comprising two non-consanguineous parents and their two affected children. The patient's symptoms were reminiscent of the non-photosensitive form of recessively inherited trichothiodystrophy (TTD). A mutation in the *TTDN1/C7orf11* gene, a gene that is known to be involved in non-photosensitive TTD, had been excluded by others by Sanger sequencing. Unexpectedly, we did find a homozygous single-base pair deletion in the coding region of this gene, a mutation that is known to cause non-photosensitive TTD. The deleterious variant causing a frame shift at amino acid 93 (C326delA) followed the right mode of inheritance in the family and was independently validated using conventional DNA sequencing. We expect this novel DNA sequencing technology to help redefine phenotypic and genomic variation in patients with (mono) genetic disorders in an unprecedented manner.

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Pollitt syndrome (MIM #275550) was first described in 1968 (Pollitt et al., 1968) for a brother and sister with mental and physical retardation and trichorrhexis nodosa (pedigree see Fig. 1). Microcephaly and abnormal cerebral cortical cell layering were associated with brittle hair having a 50% reduction in

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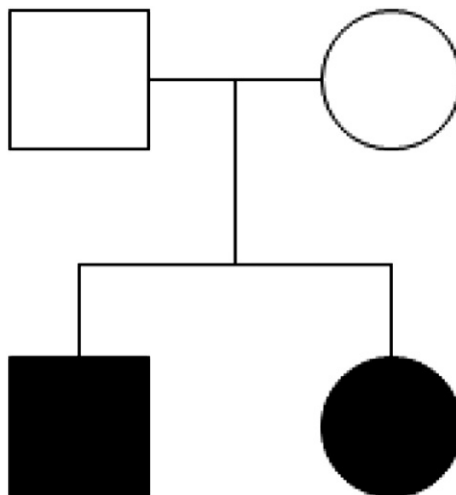


Fig. 1. Pedigree showing the two unaffected parents and their two affected children.

cysteine content, typical for trichothiodystrophy (TTD), a genetically heterogeneous autosomal recessive disorder (Faghri et al., 2008; Stefanini et al., 2010). About 50% of TTD patients are hypersensitive to UV radiation, either clinically or *in vitro* (cell cultures), consistently associated with reduced amounts of TFIIH (Botta et al., 2002; Vermeulen et al., 2000), a basal transcription factor complex with an essential role in nucleotide excision repair (Andressoo et al., 2006; Stefanini et al., 2010). The reduced amounts of the essential TFIIH factor are due to enhanced instability of the 10 subunit multi-functional complex. Biallelic mutations in one of three of its 10 known subunits (usually ERCC2/XPD, and more rarely ERCC3/XPB or GTF2H5/TTDA) are responsible (Giglia-Mari et al., 2004). Among the remaining 50% of non-photosensitive TTD cases, a minority of about 10% carries biallelic mutations in *TTDN1*, a ubiquitously expressed gene (Botta et al., 2007). The encoded protein product is suggested to play a role in maintenance of cell cycle integrity through an interaction with Polo-like kinase-1 (Plk1) (Zhang et al., 2007).

Our detailed analysis of cultured fibroblasts from the above-mentioned Pollitt syndrome family had shown fully normal nucleotide excision repair capabilities in cultured fibroblasts as well as normal TFIIH levels, clearly indicating that the sibs fall in the category of non-UV-sensitive TTD. Furthermore, mutations in the known TTD-causative genes ERCC2, ERCC3 and GTF2H5 and all other TFIIH subunits, were excluded by conventional cDNA sequencing. Remarkably, only one of the two patients carried a *de novo* heterozygous missense mutation (p.S578N) in a fully conserved amino acid of the *XPD* gene, which was apparently not related to the pathology.

After identification of the *TTDN1* gene as a cause of non-photosensitive TTD, Nakabayashi et al. (2005) reported absence of mutations in *TTDN1* in this family with Pollitt syndrome, although all the hallmarks of TTD are present. Therefore, we decided to use whole-genome diploid sequencing with the aim to identify a new TTD-causing gene.

We have used a sequencing-by-ligation method from Complete Genomics (software version 1.10.1.31, format version 1.5) as described by Drmanac et al. (2010). Briefly, the human genome sequencing procedures include DNA library construction, DNA Nano-Ball (DNB) generation, DNB array self-assembling, cPAL-based sequencing, imaging, image data analyses including base calling, DNB mapping, and sequence assembly. Reads were mapped to the National Center for Biotechnology Information (NCBI) reference genome, build 36. Variants were annotated using NCBI build 36.3 and dbSNP build 130. Data were provided as lists of sequence variants (SNPs and short indels) relative to the reference genome. Analysis of the whole genome sequencing data was performed using Complete Genomics analysis tools (cga tools version 1.3.0 build 9; <http://www.completegenomics.com/sequence-data/cgtools/>) and TIBCO Spotfire version 3.3.1 (<http://spotfire.tibco.com/>).

Mapped sequences of the four samples (father, mother and two affected children) varied between 180 and 191 Gb, resulting in an average coverage between respectively 63 and 67-fold per genome. Initially, a homozygous recessive disease model was tested. In total 5,402,954 variants were detected in one or more family members, including single nucleotide variants and small insertions, deletions and substitutions (up to about 50 bp). We searched for homozygous recessive non-synonymous variants and variants present in splice sites.

Unexpectedly, one of the only three candidate genes was *TTDN1*, showing a homozygous 1-bp deletion in codon 93 of exon1, causing a frame shift and expected protein truncation after 92 residues (p.Ser93Profs*60). Gene coverage in this region (per base, for 5 bases on either side of the deletion) ranged from 45 to 73× for the affected children and from 33 to 54× for both parents. The 1-bp deletion was not present in the HuVariome database (Stubbs et al., 2012, <http://huvariome.erasmusmc.nl/>), nor in the 1000 genomes project (<http://www.1000genomes.org/>), or in the Exome Variant Server (<http://evs.gs.washington.edu/EVS/>). The presence of this mutation that had not been identified by Nakabayashi et al. (2005) was confirmed by conventional sequencing of exon1. The finding of homozygosity was equally unexpected, since the parents (both carriers) appear not consanguineous. Further analysis revealed the identity and homozygosity of all sequence variants in the 10 Mbp region spanning the *TTDN1* gene copies of the patients. Likely, the mutation originates from a common founder, since it was also reported in two other non-photosensitive and not related TTD patients from Italy and the Netherlands (TTD31PV and TTD11RO, Botta et al., 2007). Finally, the presence and *de novo* nature of the unrelated XPD variant S578N was confirmed. Our data demonstrate that the prototype Pollitt syndrome is a regular representative of the genetic *TTDN1* class of non-photosensitive TTD. The disease of these two patients is slightly more severe than in the other reported patients with the same mutation; however, a clear genotype–phenotype correlation is missing in this class (Botta et al., 2007).

We conclude that this powerful genomics technology is readily applicable to defining phenotypic and genomic variation in patients with (mono) genetic disorders.

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