A homozygous *CREB3L1* missense mutation expands the mutational spectrum of *CREB3L1*-related osteogenesis imperfecta

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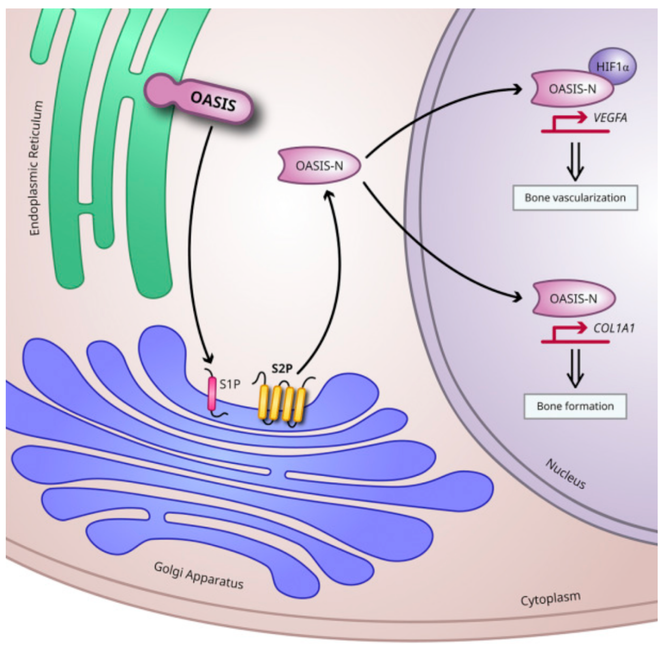
Figure 1: Working mechanism and protein function of the tissue-specific transcription factor OASIS.[1]



Background

Osteogenesis imperfecta (OI) comprises a heterogeneous group of disorders characterized by bone deformities, low bone mass, brittle bones, and connective tissue manifestations. Dominant mutations in the *COL1A1* or *COL1A2* genes account for more than 80% of the cases, whereas recessive defects can be found in a plethora of genes. In 2013, our group identified *CREB3L1* (encoding the endoplasmic reticulum (ER)-stress transducer old astrocyte specifically induced substance (OASIS)) as a novel autosomal recessive (AR) lethal/severe OI gene in a fetus in whom this gene was homozygously deleted.[2] Hitherto, only two additional homozygous mutations have been reported, an in-frame deletion c.934_936delAAG (p.(Lys312del)) and a frameshift mutation c.1365del (p.(Pro458Argfs*25)) in a family with severe AR OI.[3,4]

OASIS and its role in ER stress

In the absence of ER stress, OASIS is expressed at the ER membrane, with cytosolic N-terminal DNA-binding and transcription activation domains. In regulated intramembrane proteolysis (RIP), OASIS is transported from the ER to the golgi membrane, where it is sequentially cleaved by the endopeptidases S1P and S2P. Finally, the N-terminal domain of OASIS (OASIS-N) is translocated to the nucleus where transcriptional activation of the *COL1A1* promoter can occur.

In addition, Keller et al demonstrated that OASIS regulates the expression of the COPII component SEC24D.[3] Together with SEC23A, this protein forms the inner coat of COPII vesicles, complexes which are involved in the trafficking of secreted proteins (such as type I collagen) from the ER to the golgi.

Rationale

We report the first homozygous *CREB3L1* missense mutation associated with lethal AR OI, and investigated its pathogenicity in relation to *SEC24D/SEC23A* expression and *COL1A1* transcription.

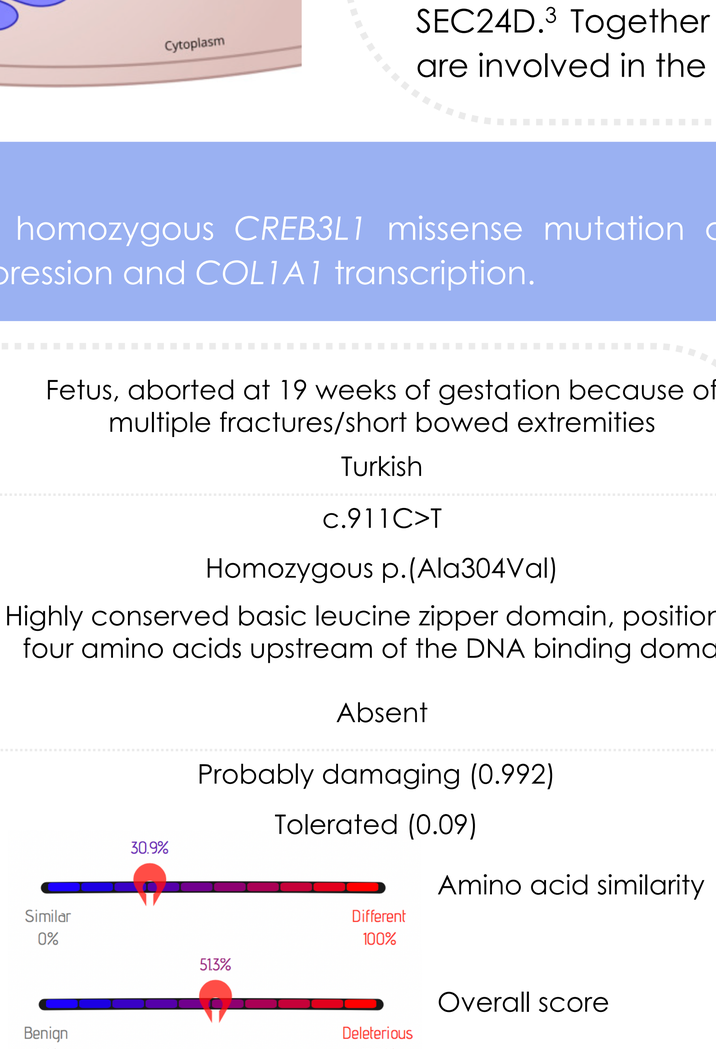
Clinical phenotype	Fetus, aborted at 19 weeks of gestation because of multiple fractures/short bowed extremities
Ethnicity	Turkish
cDNA change	c.911C>T
Protein change	Homozygous p.(Ala304Val)
Protein domain	Highly conserved basic leucine zipper domain, positioned four amino acids upstream of the DNA binding domain
Population frequency (gnomad)	Absent
PolyPhen	Probably damaging (0.992)
Sift	Tolerated (0.09)
DEOGEN2 (mutation effect prediction tool for proteins) [5,6]	 Amino acid similarity: 30.9% (Similar 0% – Different 100%); Overall score: 51.3% (Benign 0% – Deleterious 100%)

Figure 2: Clinical phenotype and molecular findings.

Conclusion

We successfully used an overexpression model to study the pathogenic nature of the homozygous missense variant p.(Ala304Val).

Quantitative reverse transcription polymerase chain reaction shows an effect on the expression of both COPII components SEC23A and SEC24D, whereas Western blot levels only link SEC24D to the *CREB3L1*-related disease mechanism.

In addition to these findings, which are in line with an earlier report [3], luciferase assays could show that the variant might also alter *COL1A1* transcription, thereby influencing the formation of the secretory coat protein II complex. This is in turn postulated to disturb the OASIS-mediated secretory pathways, necessary for normal bone development.

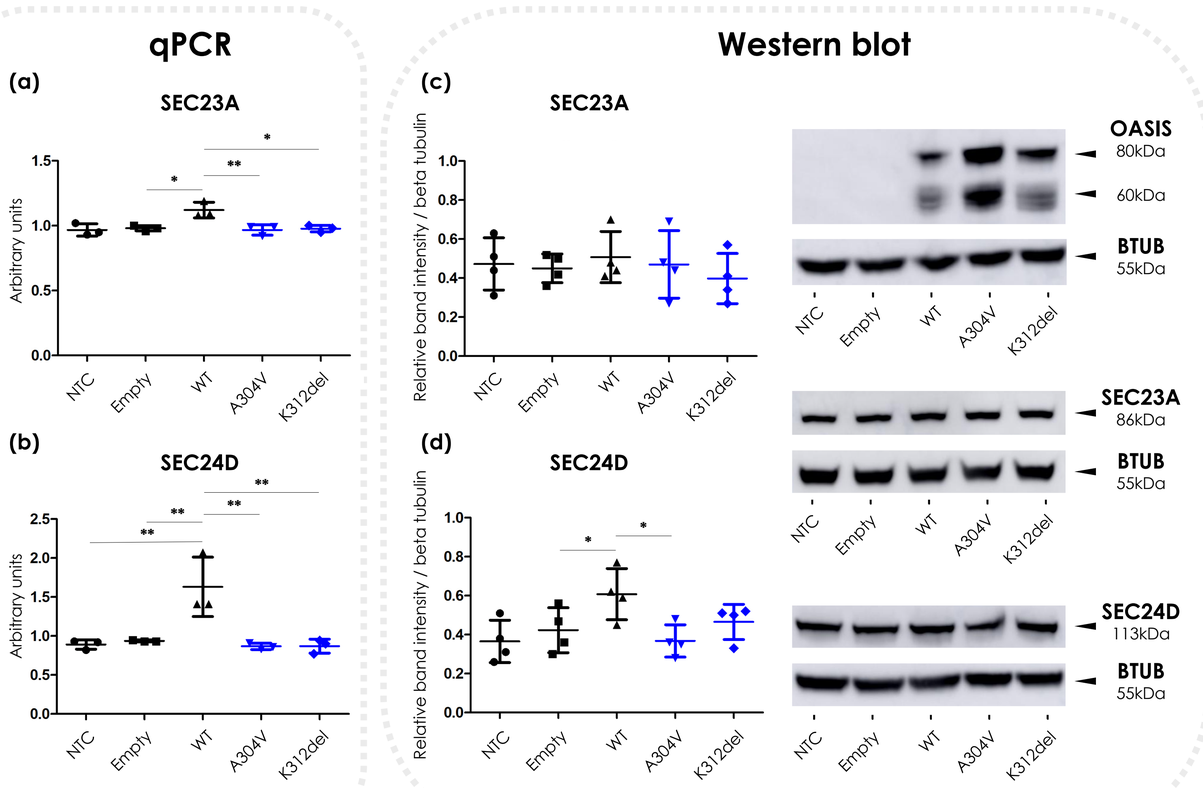


Figure 3: qPCR and Western blot results of transient overexpressed HEK293 cells.

(a) and (b): qPCR shows that both variants A304V (p.(Ala304Val), reported here) and K312del (p.(Lys312del), included as a positive control [3]) have an effect on the expression of the COPII components SEC23A and SEC24D, when compared to WT overexpressed *CREB3L1* (Tukey test, * $P < 0.05$, ** $P < 0.05$; values shown are the mean of three independent experiments (n = 3); NTC, non transfected cells; Empty, empty vector–transfected control; WT, wild type-transfected OASIS).

(c) and (d): Western blot shows that the newly identified missense variant A304V has an effect on the protein level of SEC24D, but not on SEC23A (Tukey test, * $P < 0.05$; values shown are the mean of four independent experiments (n = 4)).

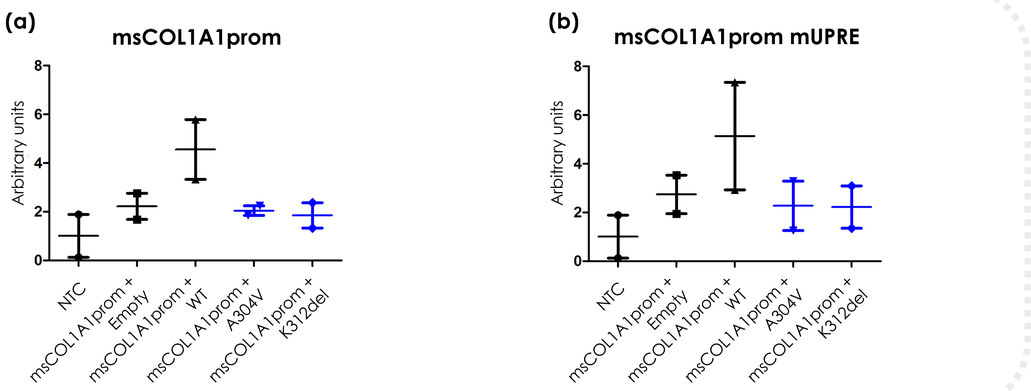


Figure 4: Luciferase assays of transient overexpressed HEK293 cells.

Luciferase assays, in which both variants A304V (p.(Ala304Val), reported here) and K312del (p.(Lys312del), included as a positive control [3]) were overexpressed together with ms *COL1A1* promoter (a) or ms COL1A1 promoter mUPRE (upstream regulatory elements) (b) constructs, indicate a possible negative effect of the transcriptional capacity of both variants. Statistics were not yet possible as these graphs display results of only 2 independent experiments (n = 2) (NTC, non transfected cells; Empty, empty vector–transfected control; WT, wild type-transfected OASIS).

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