Diamine oxidase isoforms in placenta: structural analysis and implication in pre-eclampsia

Diamine Oxidase Isoforms in Placenta: Structural Analysis and Implication in Pre-eclampsia

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Introduction

Diamine Oxidase (DAO) activity declines while histamine levels increase in pre-eclampsia (PE) (Brew and Sullivan, 2006). Two DAO isoforms (P19801-1 & P19801-2) have been identified in human placenta, where isoform P19801-2 was classified as non-functional (Zhang et al., 1995). This study used high throughput NGS technology and bioinformatics analysis to (1) determine if DAO isoform P19801-2 production in placenta could be associated with PE, and (2) whether differences in post-transcriptional conformation of DAO protein could contribute to the diminished activity in PE.

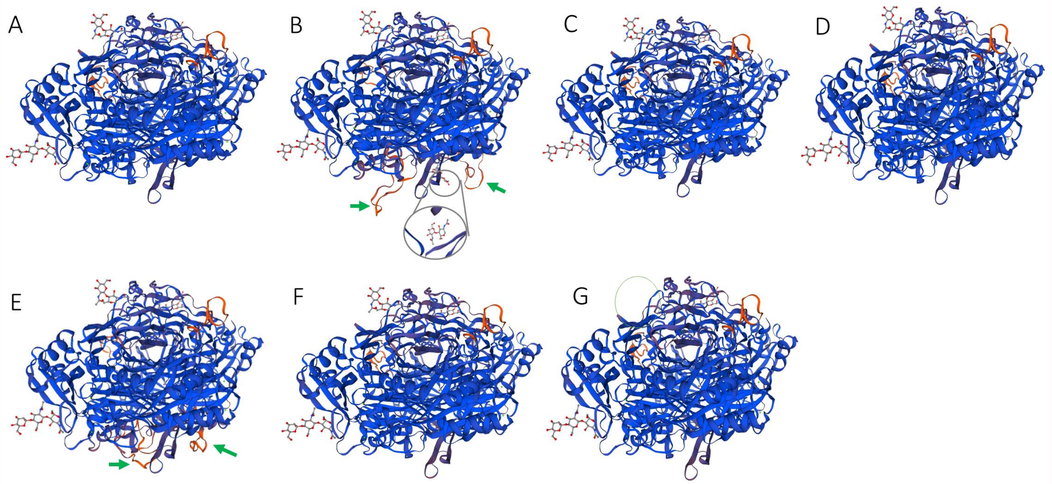

Figure 1: Tertiary Structure of DAO isoforms
Figures 1A and 1B are reference DAO structures respectively for isoforms P19801-1 & P19801-2. Figures 1C and 1D are typical DAO structures respectively for NP and PE placentae. Three novel DAO types were found in placenta (Figures 1E, 1F, 1G). Figure 1E is similar in structure to isoform P19801-2 (Figure 1B) but lacks NAG-NAG 2 ligand typical of isoform 2. Figures 1F and 1G both have 152 AA insertions at position Methionine 1 to Glutamic acid 152 and share structure similarity with isoform P19801-1 (Figure 1A), but Figure 1G lacks NAG-NAG 3 ligand bonding.

Green arrows = 19 AA insertion at positions Alanine 618 to Glycine 637. Figure 1B insert = NAG-NAG 2 ligand site typical of DAO isoform 2

Methods

RNA-Seq raw data from a total of 84 pregnancies (NP = 55; PE = 29) that met inclusion criteria were obtained from NCBI SRA. Sample quality was assessed with Fastqc and all samples has phred scores of >20. 0% failed MAPQ from Star aligner. AA sequence conservation in DAO was analysed with von Neumann entropy in PFAAT on MUSCLE. Sequence phylogeny was examined with MEGA-X, Protein hydrophobicity, 3D structure, protein bonds, torsional angles and Ramachandran plots were modelled with SWISS-MODEL.

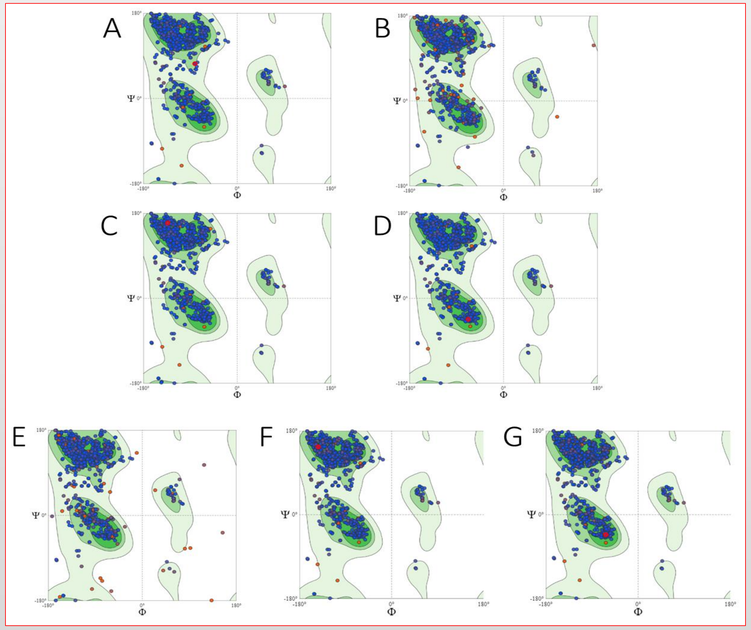

Figure 2: Ramachandran Plots (RP) of DAO proteins
Figure shows phi-psi (ϕ-ψ) torsion angles of the protein backbone in DAO protein. Figures 2A & 2B shows RP for DAO reference Isoforms 1 and 2 respectively. Figures 2C & 2D shows RP respectively for NP and PE placental DAO Figures 2E, 2F and 2G shows RPs for new variants 1 – 3, respectively. RP for Figure 2E is akin to Figure 2B, confirming that Figure 2E is an isoform 2 type. Figure 2E was obtained from a normal placenta with normal DAO activity, indicating that Figure 2E is a functional DAO.

Table 1: Occurrence of Amino Variations in PE and NP DAO proteins

Amino Acid Variants	NP DAO (%)	PE DAO (%)
Non-conserved Beta-sheet	82 (64)	47 (36)
Non-conserved Loop	14 (58)	10 (42)
Conserved Beta-sheet	0 (0)	1 (100)
Conserved Loop	2 (40)	3 (60)

Total of 162 amino acid variants were found in 84 protein sequences (NP = 55; PE = 29). About 2-fold less AA variations were found in PE than in NP samples. However, more Threonine and Serine residues were substituted for Methionine and Phenylalanine in PE than in NP proteins. Thus, rending PE proteins more hydrophobic than NP placental proteins.

Conclusion

Three types of DAO proteins are found in placentae.

- All 3 subtypes were found in mothers with no clinical evidence of diminished DAO and PE.
- One subtype is structurally similar to DAO isoform 2, with a prevalence rate of 1.2%.
- Two other subtypes found have additional 152 AA insertion, preserved structural similar to DAO isoform 1; and occurs at a prevalence rate of 3.6%.
- Isoform 2 like protein found in placentae is functional

References:
Brew O, Sullivan MH. The links between maternal histamine levels and complications of human pregnancy. J Reprod Immunol. 2006 Dec;72(1-2):94-107
Zhang., et al. (1995a) 'cDNA sequences of variant forms of human placenta diamine oxidase', *Biochemical genetics*, 33(7-8), pp. 261-268.