Antley-Bixler syndrome or POR deficiency?

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Abstract

Antley-Bixler syndrome (ABS) is a rare congenital disorder characterized by numerous craniofacial, skeletal and, in some cases, urogenital abnormalities resulting from disordered steroidogenesis. Known genetic causes in sporadic cases of ABS include dominant mutations in the fibroblast growth factor 2 receptor gene (FGFR2). Recent research shows surprisingly that symptoms of Antley-Bixler syndrome, combined with disordered steroidogenesis and urogenital anomalies, are caused by mutations in the POR gene that encodes NADPH-cytochrome P450 oxidoreductase (CYPOR).

CYPOR is a four domain-containing monomeric flavoprotein that contains two flavins, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), a binding site for NADPH, and the N-terminal sequence of 25 amino acids which determines the microsomal localization of the protein. CYPOR is the electron donor to microsomally localized cytochromes P450 that participate in xenobiotic metabolism and steroidogenesis. Mutations in the POR gene lead to apparent diminished activity of some P450 enzymes.

Association of CYPOR with ABS discloses new facts about this disease and recent research shows that patients with ABS-like skeletal anomalies, but with mutations in the POR gene and disordered steroidogenesis, represent a new disorder called POR deficiency.

Key words: Antley-Bixler syndrome, POR deficiency, NADPH-cytochrome P450 oxidoreductase, disordered steroidogenesis.

Clinical features of Antley-Bixler syndrome

Antley-Bixler syndrome (ABS, OMIM 207410) is a rare but very serious disorder characterized by numerous craniofacial, skeletal and urogenital abnormalities. The first case of ABS was described in 1975 (1). ABS primarily manifests with brachycephali, sever mid-

face hypoplasia, craniosynostosis, radiohumeral synostosis, femoral bowing and spontaneous long-bone fractures. These malformations appear in the majority of cases described to date (approximately 70 described cases). Other manifestations comprise choanal atresia as well as stenosis, loss of hearing, agenesis of kidneys, proptosis, arachnodactilia, heart malfomations and, in some patients, disordered steroidogenesis and ambiguous genitalia. Many patients suffer early death, though in recent years several long–term survivors with normal life spans have been described (2-4). Early death was reported in approximately half of known cases, usually due to respiratory complications (5). Development of intelligence of affected individuals is probably dependant on two main factors, which are craniosynostosis and obstruction of upper airways (6). It seems that early and efficient management of these complications are prerequisite for healthy mental development of the patient.

Etiology of ABS

The etiology of ABS has been considered heterogeneous. At the beginning ABS was suggested as a disorder with an autosomal dominant mode of inheritance. This presumption was based on three cases of ABS which occurred in siblings (7-9) and two cases of ABS born to consanguineous couples (10, 11). Although Suyuki´s siblings (9) and Yasuia´s children of the consanguineous couple (10) are those same cases, the rest of described patients occurred sporadically, therefore there was need to search for other possible causations of the disease.

In 1998 Chun et al. (12) reported a child with anomalies characteristic for ABS who carried dominant *de novo* mutations in the fibroblast growth factor receptor 2 (FGFR2) gene. Authors proposed that ABS is an autosomal dominant condition with possible gonadal mosaicism. Although there has been objection to the ABS classification of the patient described by Chun et al. (13, 14), association of the craniosynostosis and elbow ankylosis with dominant de novo mutation in the FGFR2 has been proved (15).

New insight into the etiology of ABS was gained from the discovery that an ABS-like phenotype occurred in an infant born to a woman treated with fluconazole (16-18). Fluconazole is an antifungal antibiotic that inhibits biosynthesis of ergosterol in the fungal cell-wall. The primary mechanism of action of fluconazole is the inhibition of lanosterol-14-alpha-demethylase (CYP51A1). This enzyme inhibits lanosterol demethylation which leads to depletion of ergosterol in the cell-wall. In mammalian cells CYP51A1 plays an important role in cholesterol synthesis. Teratogenic effects of fluconazole have not been proved in further studies (19), but this discussion and abnormal growth of certain patients´ genitals impel researchers to study steroid metabolism in effected individuals.

An important breakthrough in understanding the pathogenesis of ABS occurred when Reardon et al. (20) reported alteration of steroid biogenesis in 7 of 16 patients with ABS, one of whom carried FGFR2 mutation. Disruptions of the steroid metabolism in that series were not so severe and five of the females had ambiguous genitalia. The report on abnormalities in steroidogenesis indicated insufficiency of the enzyme 21-hydroxylase (CYP21A2), but DNA analysis of CYP21A2 gene did not reveal any mutation. This report first referred to the fact that some cases of ABS are outcomes of two distinct genetic events, concretely, outcomes of mutations in the FGFR2 gene and in some gene causing alteration in biosynthesis of steroid hormones. Other reports on abnormalities in sterol biosynthesis followed from that work (2, 21). After excluding mutations in genes encoding the apparently impaired steroidogenic enzymes (lanosterol-14-alpha-demethylase, 17-alpha-hydoxylase, 21-hydroxylase) (2, 20, 21) in observed patients, mutations in POR gene, encoding NADPH-cytochrome P450 oxidoreductase (CYPOR) were identified as the cause of ABS (22-24). CYPOR is the main electron donor to each of the steroidogenic enzymes mentioned above.

Miller´s group (22) discovered six allelic variants of the POR gene (R457H, V492E, A287P, C569Y, V608F and an intron 6 splice variant resulting in a premature stop codon) in four unrelated patients with disordered steroidogenesis. Among that group, three were ABS patients and none of them carried a mutation in the FGFR2 gene. Arlt et al. (25) identified another POR mutation (Y181D) by analysis of the POR gene in patients with congenital adrenal hypoplasia. Several new POR variants were identified among Japanese patients. They included insertion mutations (I444fsX449 and L565fsX574) (23), a deletion mutation (L612_W620delinsR), a silent mutation (G5G) and a missense mutation (Y578C) (3). All patients had disordered steroidogenesis. Both of Adachi`s patients (23) were compound heterozygotes in the POR gene and did not carry any mutation in the FGFR2 gene. Fukami`s group (3) reported that nine of their ten patients were found to by homozygotes or compound heterozygotes for five types of POR mutations. One case carried a mutation just in one allele. They did not carry out the analysis of the FGFR2 gene. Subsequently, 11 new missense mutations (A115V, T142A, Q153R, M263V, Y459H, A503V, G539R, L565P, R616X, V631I and F646del) were discovered in a study in which 32 ABS patients were queried to address the distribution of POR and FGFR2 variants (24). Sequencing of both genes showed complete segregation of POR and FGFR2 mutations. Fifteen patients carried POR mutations on both alleles, 4 carried mutations on only one allele, 10 carried FGFR2 or FGFR3 mutations, and 3 patients carried no mutations. These findings definitely confirmed the digenic origin of ABS

and the contribution of POR mutations to the development of the syndrome. Most recently, Homma et al. described three new POR variants (Q201X, A462_S463insIA and E580Q) (26).

In February last year another protein occurred to play a role in the etiology of ABS. Hughes et al. (27) reported on a microsomally localized hemoprotein PGRMC1, that forms a stable complex with several members of the cytochrome P450 family of enzymes and positively regulates their activities. PGRMC1 is the third protein (beyond CYPOR and cytochrome b_5) known to interact with and modulate the activity of microsomal cytochromes P450.

NADPH-cytochrome P450 oxidoreductase

The enzyme NADPH-cytochrome P450 oxidoreductase is a membrane binding flavoprotein that contains two flavins, FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide). The main function of the CYPOR protein is to transfer electrons from reduced nicotinamide adenine dinucleotide phosphate (NADPH) to all microsomal cytochrome P450 enzymes (Fig. 1) and at the same time to other acceptors such as heme oxygenase (28), fatty acid elongase (29), squalene epoxidase (30) or cytochrome b_5 (31). By interacting with microsomally located cytochromes, CYPOR participates in xenobiotic and drug metabolism and steroidogenesis. By other biochemical pathways, it participates in metabolism of prostaglandins and fatty acids.

The enzyme NADPH-cytochrome P450 oxidoreductase was first isolated and purified by Horecker in 1950 from liver cells (32). These early studies did not permit the localization of the enzyme to any subcellular compartment. In 1956 Strittmatter and Velick (33), while studying NADH activities, discovered a separate microsomal fraction that catalyzed cytochrome *c* reduction 20 times faster with NADPH than with NADH, which they proposed could be the enzyme described previously by Horecker. Microsomal localization of CYPOR was confirmed at the same time by Williams and Kamin (34) and by Phillips and Langdon (35). Another important event in the history of CYPOR discoveries was the finding that the enzyme is a flavoprotein that contains both FAD and FMN (36) and not just FAD, as discovered by Horecker. Shephard et al. (37) isolated and sequenced rat and human CYPOR. By Southern blot analysis of DNA, Shephard et al. (37) determined that CYPOR is encoded by a single gene located on the long arm of the 7th chromosome and by *in situ* hybridization they refined localization to 7q11.23. The POR gene contains 15 exons. Finally, the rat CYPOR crystal structure revealed that the enzyme acts as a monomer and contains four

domains (38) (Fig. 2A). A sequence of 25 amino acids in the N-terminal part of the protein determines the microsomal localization of the protein and its linkage to the membrane.

Phenotype manifestations of different POR mutations vary. It has been proved that POR knockout mice are embryonic lethal (39). POR mutations in ABS patients are probably less severe than total gene deletion since they provide some degree of residual CYPOR activity.

Disruption of CYPOR function leads to apparent insufficiency of CYPOR-dependent enzymes, CYP17A1 (17-alpha-hydoxylase and 17, 20-lyase), CYP21A1 (21-hydroxylase) and CYP51A1 (lanosterol-14-alpha-demethylase). It was proved that some POR mutations cause diminished affinity of affected proteins for the essential FAD cofactor (40). In this situation, the important coenzyme-cofactor relationship is impaired such that CYPOR can not transfer electrons to its acceptors. Consequently, cytochromes P450 can not function normally and appear deficient. As shown in Fig. 3, several steps in the pathway of steroidogenesis are inhibited by depressed activity of these enzymes.

The effects of POR mutations on the development of skeletal anomalies remain unclear. Since skeletal malformations are observed in embryonic lethal POR knockout mice (39), it is possible to conclude that disruption of CYPOR function may be responsible for skeletal abnormalities in ABS patients. This theory was first suggested by Flück et al. (22). It has also been shown that mouse embryos with disrupted cytochrome CYP26A1 (a CYPOR-dependent enzyme) undergo defects in vasculogenesis and head development (41). CYP26A1 plays a role in retinoic acid (RA) metabolism and it is required to prevent teratological effects that may result from RA signaling. Presently, the focus is on cholesterol metabolism. It is obvious that disorders involving enzyme defects in cholesterol biosynthesis include skeletal malformation phenotypes (42). Synthesis of cholesterol relies on two CYPOR-dependent enzymes, squalene monoxygenase and CYP51A1. It is hypothesized that anomalies may develop due to reduced activity of CYP51A1 (43), but the main mechanism remains to be determined.

Conclusion

It is important to realize that the clinical picture of patients with POR mutations vary. It is assumed, that POR mutations are more common than it is suggested by the relatively low incidence of ABS and that milder mutations could result in disordered steroidogenesis, increased sensitivity to drugs and environmental toxins (22) or in the milder syndrome manifestation. Occurrence of milder types of the syndrome confirms some studies (4). Most

described POR mutations are found in the NADPH, FAD and FMN co-factor binding sites and functionally important domains of CYPOR (Fig. 2B), while apparent polymorphisms are found in regions with lesser structural importance (24, 25). Evidently, different POR mutations lead to different phenotypes, therefore it is very important to study genotype-phenotype correlations. Insight into the etiology of ABS has brought about new terminology. The term ABS is now used to indicate individuals with the ABS-like phenotype and normal steroidogenesis, whereas those with skeletal and craniofacial malformations, ambiguous genitalia, disordered steroidogenesis and mutations in the POR gene are recognized as having a distinct new disease: POR deficiency (24, 26, 44). Apparently, further research is required to understand the genetic and biological bases of the disorder and to address the effects of different polymorphisms on the phenotypes of patients.

List of used abbreviation

ABS - Antley-Bixler syndrome

CYP17A1 - 17-alpha-hydoxylase

CYP21A2 - 21-hydroxylase

CYP26A1 - cytochrome P450 retinoic acid-metabolizing enzyme

CYP51A1 - lanosterol-14-alpha-demethylase

CYPOR - NADPH-cytochrome P450 oxidoreductase

FAD - flavin adenine dinucleotide

FGFR2 - fibroblast growth factor 2 receptor

FMN - flavin mononucleotide

POR - gene for NADPH-cytochrome P450 oxidoreductase

RA - retinoic acid

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Figure 1. Schematic representation of the electron transport from CYPOR to microsomal cytochrome P450 enzymes.

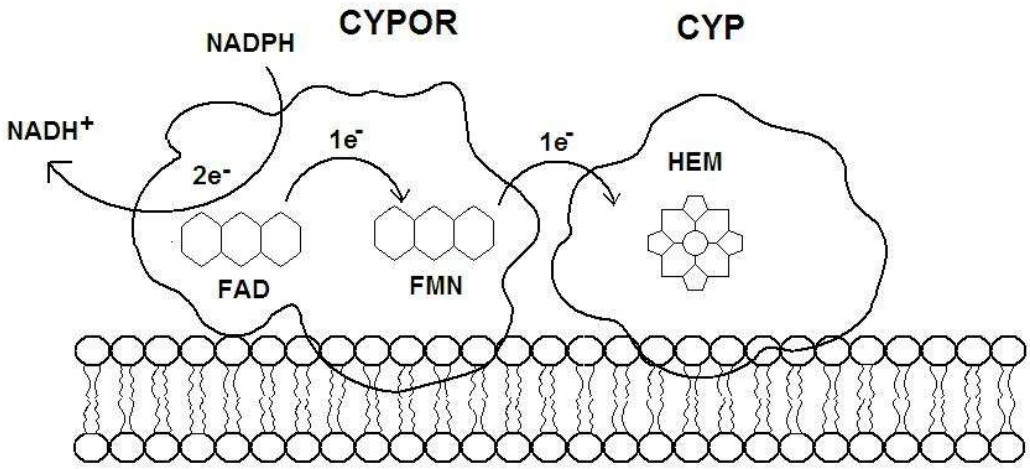

Figure 2. The structure of NADPH-cytochrome P450 oxidoreductase from rat (37) sharing ~95% sequence identity with human. Panel A – Ribbon diagram of the backbone structure with N' and C' termini labeled, FMN-binding domain shown in blue, connecting domain in green, FAD-binding domain in yellow, and NADPH-binding domain in pink. Panel B – Variant residues in proximity to FMN, FAD, and NADPH are drawn in stick configuration. Variants 1-4 (black) correlate with the severe craniofacial ABS phenotype, while 5-6 (gray) correlate with disordered steroidogenesis (22, 25).

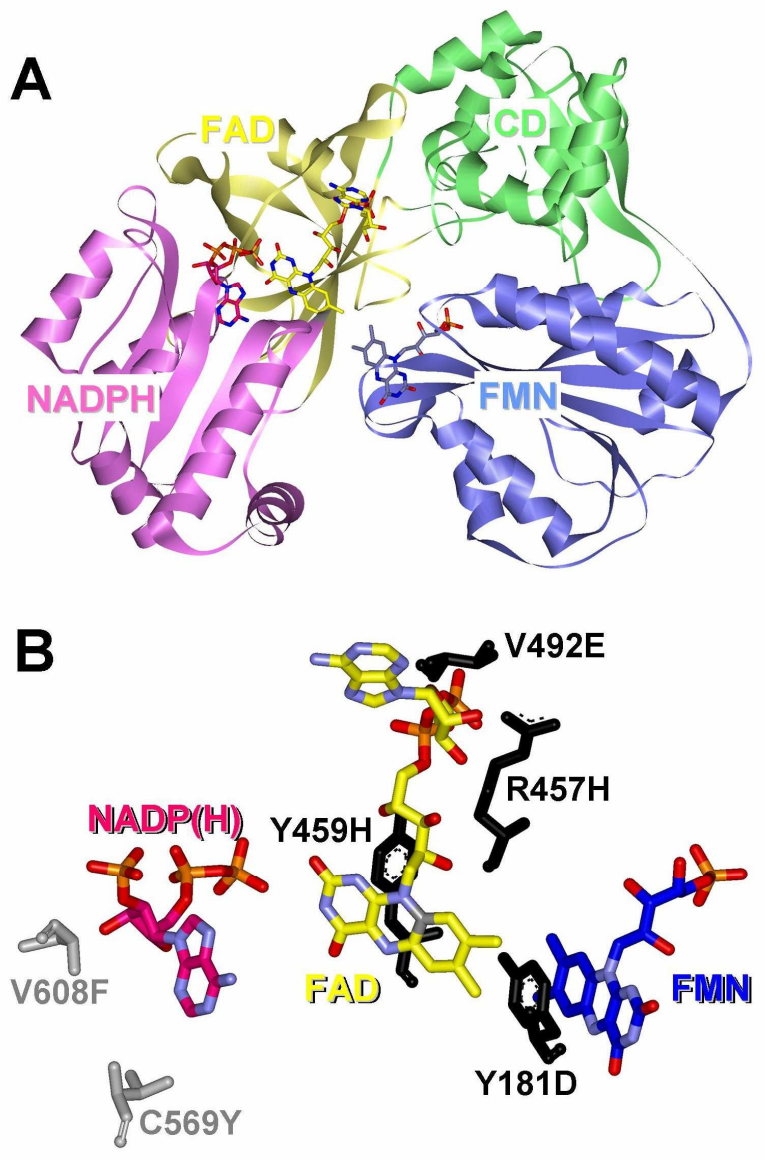

Figure 3. Schematic representation of impaired steroidogenesis in POR mutations.