Evidence type	**Evidence Combinations:** Each column indicates the criteria required to reach supporting or moderate PP4 strength level							
	Supporting					**Moderate**		
Biological sex at birth	Male			Female		Male	Female	
Clinical Findings **A.** Primary adrenal insufficiency	A OR B OR C	A	NR (*)	B	A[¥] OR C[¥] OR NR (*)	A OR B OR C	NR	NR (*)
B. Myelopathy with or without peripheral neuropathy (adrenomyeloneuropathy, AMN) **C.** Rapidly progressive inflammatory white matter demyelination develops in childhood (childhood cerebral ALD; CCALD), adolescence (adolescent cerebral ALD) or, less frequently, adulthood		B OR C					B[€]	
Family History **D.** Family members affected with any of the described phenotypes, with an X-linked inheritance pattern (within a family, different phenotypes, including just elevated VLCFAs, can be present). At least one family member affected should be a male	NR	D	D OR E	NR	D	D OR E	D OR E	D AND E
E. Panel-based testing OR exome/genome excluding other peroxisomal disorders[#] *If *ABCD1* is sequenced as a single-gene test, exclusion of the 4 pseudogenes (on chromosomes 2, 10, 16 & 22) is assumed.								
F. Diagnostic Testing (Abnormal results for any ONE of the following is sufficient) Abnormal biochemical genetic testing: increased plasma or serum C26:0 with elevated C24:0 to C22:0 and C26:0 to C22:0 ratios (VLCFA testing). Indicating "abnormal" test result is sufficient (specific values are not necessary) -OR- Abnormal biochemical genetic testing: increased C26:0-lysophosphatidylcholine and ratios. Indicating "abnormal" test result is sufficient (specific values are not necessary) -OR- Decreased beta-oxidation activity/absence of ALDP on immunoblot/mislocalization by immunofluorescence in patient cells (fibroblasts)	F	NA (**)	F	F	F	F	F	F

NR: Not Required; NA: Not available

(*) Asymptomatic patient

(**) Biochemical genetic testing results are NOT reported/available. Does NOT apply to negative results.

[¥]Very rare in females as an isolated phenotype

[€]Adult patient

[#]*ACOX1*, *HSD17B4*, *ACBD5,* and all *PEX* genes except *PEX7*.

Notes

- Primary adrenal insufficiency is characterized by low cortisol production despite high levels of adrenocorticotropic hormone (ACTH). The lifetime prevalence in males with ABCD1 deficiency is ~80% (ref. 1); onset is typically in the first decade of life. Clinical signs and symptoms include hyperpigmentation, failure to thrive, hypoglycemia, weakness, and unexplained nausea/decreased appetite. The signs of mineralocorticoid deficiency (salt cravings, polyurea) usually present later. Adrenal insufficiency is rare in women carrying *ABCD1* pathogenic variants (approximately 1% of patients). Adrenal insufficiency has also been reported in patients with Zellweger spectrum disorder
- Myelopathy with or without peripheral neuropathy (adrenomyeloneuropathy, AMN) manifests as slowly progressive spastic paraparesis and sensory ataxia, with onset in the 2nd to 5th decade of life in men and usually later (5th decade) in women (ref. 1-4). Most patients eventually lose ambulation. Cervical spinal cord atrophy is evident by MRI in advanced stages. Frequently present symptoms are neurogenic bladder or fecal incontinence, the latter being a frequent and early sign in women (ref. 3) Approximately 70% of males with AMN will also develop adrenal insufficiency. AMN is the most common clinical presentation in women, with approximately half of them developing myelopathy and/or peripheral neuropathy over time
- Rapidly progressive inflammatory white matter demyelination develops in childhood (childhood cerebral ALD; CCALD) or adolescence (adolescent cerebral ALD). Cognitive deficits and behavioral problems are often the first symptoms, and are attributed to other conditions, such as attention deficit hyperactivity disorder (ref. 1-2). If untreated, patients rapidly develop severe cognitive and motor disability. Death occurs on average 2 years after the onset of symptoms. White matter lesions by MRI precedes symptoms. Cerebral ALD in women is exceedingly rare (ref.3-4)
- Approximately 20% of women carrying an *ABCD1* pathogenic variant have normal VLCFA results in plasma/serum (evidence E), even when symptomatic. Recent data have shown a much higher sensitivity in detecting ALD heterozygotes by using C26:0-lysophosphatidylcholine in dried blood spots or plasma (evidence F; ref. 5-7)
- Abnormal biochemical genetic testing is present in patients with Zellweger spectrum disorder (ZSD) and other peroxisomal disorders, although usually the overall clinical presentation is suggestive of ABCD1 deficiency
- If the single gene is sequenced, exclusion of the 4 pseudogenes (on chromosomes 2, 10, 16 & 22) is assumed to be confirmed (Information on *ABCD1* pseudogenes has been available since 1997, PMID: 9215666; more data in the Dutch database, PMID: 35053399). Sequencing labs are aware of the existence of pseudogenes and are routinely expected to exclude them in analysis. While this information is not required for the application of the PP4 criteria, it is essential to note in curations when available.

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