THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Genetic Testing for Robinow Syndrome and Brachydactyly, type B1

Clinical Features:

- Patients with Robinow syndrome (RS) [OMIM #268310] have characteristic facial features, growth retardation, limb defects, and genital hypoplasia. Skeletal anomalies include mesomelic or acromesomelic limb shortening, hemivertebrae, and brachydactyly. Characteristic facial features include macrocephaly, prominent broad forehead, hypertelorism, midface hypoplasia, short upturned nose with depressed nasal bridge and flared nostrils, large triangular mouth, micrognathia, and low-set ears. Approximately 10-15% of individuals have developmental delay. Other features include heart defects (~15%), renal tract anomalies, rib fusions and nail hypoplasia or dystrophy. Approximately 10% of children with RS have an early fatal outcome due most likely to congenital heart defects. Patients with autosomal recessive RS (RRS) appear to be more severely affected than those believed to have autosomal dominant RS (DRS). Vertbral anomalies, radial head dislocations and scoliosis are rarely seen in DRS. Height is usually nearer the normal range in DRS [1,2].
- Patients with brachydactyly, type B1 (BDB1) [OMIM #113000] have hypoplasia/aplasia of the distal phalanges and nails. Middle phalanges are short and terminal phalanges are rudimentary or absent. Usually, the thumbs and big toes are spared, but may have broadening or partial duplication. BDB1 is the most severe form of brachydactyly.

Molecular Genetics:

- Autosomal recessive Robinow syndrome—Mutations in the *ROR2* [OMIM #602337] gene have been identified in patients with RRS [3,4]. Afzal AR, *et al* [2000] reported that 10/10 consanguineous families with RRS had homozygous mutations in *ROR2* [4]. Bokhoven H, et al [2000] found that 7/11 consanguineous families with RRS had homozygous mutations in *ROR2* [3]. Approximately 65-100% of individuals with RRS have mutations in *ROR2.* Missense, nonsense, and frameshift mutations have been reported in both the intracellular and extracellular domains of the ROR2 protein. These mutations are predicted to be "loss-of-function" mutations and heterozygous carriers do not typically have a clinical phenotype [5].
- Autosomal dominant Robinow syndrome - To date, two missense mutations in *WNT5A* [OMIM#164975] have been identified in patients with DRS [6]. Functional expression assays in zebrafish embryos showed that the mutant proteins represented hypomorphic alleles rather than dominant-negative mutations. Autosomal dominant Robinow syndrome is rarer than autosomal recessive Robinow syndrome. Heterozygous truncating variants in *DLV1* and *DVL3* have been identified in patients with DRS[7,8]. To date, all the reported pathogenic variants in *DVL1* have been frameshift variants in exon 14 (the penultimate exon) and are thought to lead to a truncated transcript that escapes nonsense mediated decay (Bunn et al., 2015; White et al., 2015)[9]. A gain of function or dominant-negative mechanism has been proposed (White et al., 2015). *De novo* truncating variants in *DVL3* were identified in the last exon in five unrelated individuals with DRS.
- Brachydactyly, type B1—Heterozygous mutations of the *ROR2* [OMIM #602337] gene have been identified in patients with BDB1 [10]. All of the *ROR2* mutations reported in patients with BDB1 have been truncating mutations in the intracellular domain of the protein. These mutations are predicted to be "gain-of-function" mutations. Those mutations distal to the tyrosine kinase domain cause a severe, amputation-like phenotype affecting three or more fingers, whereas proximal mutations produce a less severe phenotype [5].

ROR2 has 9 coding exons. The ROR2 protein is a member of the ROR kinase family with tyrosine kinase activity. It is highly expressed in the developing nervous system, chondrocytes, branchial arches, heart and limb buds during mouse development [3]. *WNT5A* has 5 coding exons. *ROR2* is a putative *WNT5A* receptor and the WNT5A/ROR2 signal transduction pathway it important in human craniofacial and skeletal development [6]. *DVL1* and *DVL3* genes encode human homologs of the disheveled proteins belonging to a family of intracellular scaffolding proteins and are thought to play a role in transmission of canonical and non-canonical Wnt signals. They have 15 exons each.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

Inheritance:

- Autosomal recessive Robinow syndrome—*ROR2* mutations that cause RRS are inherited in an autosomal recessive pattern. RRS is rare. It occurs more commonly in consanguineous families and those of Turkish and Omani origin. Parents of an affected child are most likely obligate carriers. Recurrence risk for carrier parents is 25%.
- Autosomal Dominant Robinow syndrome – *DVL1, DVL3, WNT5A* mutations that cause DRS are inherited in an autosomal dominant pattern. Recurrence risk for unaffected parents of an isolated case is <1%. Recurrence risk for affected individuals is 50%.
- Brachydactyly, type B1is inherited in an autosomal dominant pattern. Recurrence risk for affected individuals is 50%.

Additional Resources:

Robinow Syndrome Foundation
Phone: 763-434-1152
Karla Kruger email: robinowfoundation@comcast.net
www.robinow.org

Test methods:

Comprehensive sequence coverage of the coding regions and splice junctions of the *DVL1*, *DVL3*, *WNT5A* and *ROR2* genes is performed. Targets of interests are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All pathogenic and likely pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp. Our CNV detection algorithm was developed and its performance determined for the sole purpose of identifying deletions and duplications within the coding region of the gene(s) tested. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Regions of high homology and repetitive regions may not be analyzed. This methodology will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of our deletion/duplication assay may be reduced when DNA extracted by an outside laboratory is provided.

Please send a completed RRS/BDB1 Clinical Checklist and patient consent form with each sample.

Robinow Syndrome Panel (Sequencing and deletion/duplication analysis of *DVL1*, *DVL3*, *ROR2* and *WNT5A*)

Sample specifications:	3 to 10cc of blood in a purple top (EDTA) tube
Cost:	$2500
CPT codes:	81406, 81407
Turn-around time:	8 weeks

Results:

Results, along with an interpretive report, will be faxed to the referring physician. Additional reports will be provided as requested. All abnormal results will be reported by telephone.

References:

1. Bacino C. ROR2-Related Robinow Syndrome. In: Pagon R, Bird T, Dolan C, eds. *GeneReviews [Internet]*. Seattle: University of Washington; 2005.
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3. van Bokhoven H, Celli J, Kayserili H, et al. Mutation of the gene encoding the ROR2 tyrosine kinase causes autosomal recessive Robinow syndrome. *Nat Genet.* 2000;25(4):423-426.
4. Afzal AR, Rajab A, Fenske CD, et al. Recessive Robinow syndrome, allelic to dominant brachydactyly type B, is caused by mutation of ROR2. *Nat Genet.* 2000;25(4):419-422.
5. Afzal AR, Jeffery S. One gene, two phenotypes: ROR2 mutations in autosomal recessive Robinow syndrome and autosomal dominant brachydactyly type B. *Hum Mutat.* 2003;22(1):1-11.

6. Person AD, Beiraghi S, Sieben CM, et al. WNT5A mutations in patients with autosomal dominant Robinow syndrome. *Dev Dyn.* 2010;239(1):327-337.
7. White JJ, Mazzeu JF, Hoischen A, et al. DVL3 Alleles Resulting in a -1 Frameshift of the Last Exon Mediate Autosomal-Dominant Robinow Syndrome. *Am J Hum Genet.* 2016;98(3):553-561.
8. White J, Mazzeu JF, Hoischen A, et al. DVL1 frameshift mutations clustering in the penultimate exon cause autosomal-dominant Robinow syndrome. *Am J Hum Genet.* 2015;96(4):612-622.
9. Bunn KJ, Daniel P, Rösken HS, et al. Mutations in DVL1 cause an osteosclerotic form of Robinow syndrome. *Am J Hum Genet.* 2015;96(4):623-630.
10. Oldridge M, Fortuna AM, Maringa M, et al. Dominant mutations in ROR2, encoding an orphan receptor tyrosine kinase, cause brachydactyly type B. *Nat Genet.* 2000;24(3):275-278.

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