An introduction to the

Niemann-Pick Type C Disease Gene Variation Database

( http://npc.fzk.de )

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This short manual is supposed to give an introduction to new users of NPC-db (vs 1.1). It will guide readers through the different functions of the database and provide some basic operational instructions. In case you are interested in more detailed instructions, if you have further questions or suggestions, please contact the administrator via Heiko.Runz@med.uni-heidelberg.de.

1. The Main Page (Home)

The main-page (Home) provides access to the database. A sidebar displays options available to the User. It can be used as an entry point to directly search for sequence variation, genotypic or phenotypic information, view lists of NPC gene variants or submit novel molecular or clinical data. The main page further links to the following pages:

1.1. Aims & Scope gives general information on NPC disease and disease related topics. Motivation and aims of the database are described. This User Manual and a Manual
Data Submission Form for submission of molecular or clinical information can be downloaded as pdf-files from this page.

1.2. **Impressum** provides curator information and administrator contact details.

1.3. **Disclaimer** *(is a typical German necessity to prevent the curators from getting in trouble with authorities because of linked content)* ;-(

1.4. **Related Links** provides access to homepages of several national self support groups and patient organizations.

For general information on the genes **NPC1** and **NPC2** and further information on NPC disease, select your gene of interest in the **Select Gene** drop-down menu and click on **Show Gene information**.

This will lead to a **Gene Info**-page which provides an overview of gene-related data (e.g. chromosomal location or NCBI RefSeq-IDs of the selected gene). Links to www-resources provide further gene and disease-related information (NCBI Entrez, OMIM, GeneReviews, HGNC). By numbering the listed variations per gene and showing the submission date of the last submission, this site also provides statistics on the usage and comprehensiveness of NPC-db.

Clicking on the **number of Submitted variations until** leads to an overview of all variations submitted until a displayed date (also accessible from **Home**). A click on **Search for variations in this gene** will lead to the search option section of the Database.
2. Search options

Three different search options are implemented in NPC-db, Variation search, Genotype search and Phenotype search.

2.1. Variation search

This function allows defined searches for known genetic variants within either NPC1 or NPC2. Note that for performing your queries first a gene has to be selected (If you have entered this page via Gene Info, the gene is selected automatically). Searching for variants in both genes at once is not possible to date. A set of parameters can be specified which allow targeted sequence variation searches (e.g. by exon number, position in the protein sequence, disease association or reference). Unmarked check boxes are not considered for the query. Upon defining search parameters, the user can define the format how search results are to be displayed.

2.2. Genotype search

This option allows searches for comparison of genotype and phenotype information. From a drop-down menu, the user can select one variation of interest. After clicking Search, all listed patients with the selected variant are displayed. Importantly, this function gives information on which other alleles have been described in combination with the selected variant. As many NPC patients show compound heterozygosity in NPC1 or NPC2, this function of NPC-db is of special value for clinical evaluation of a variant in molecular diagnostic settings. Moreover, if available, additional genetic variants such as frequent single nucleotide polymorphisms (SNPs) are are listed for individual patients.
If phenotypic information has been submitted along with sequence information on an individual patient, **Show Phenotype of this patient** is displayed in the left column of the results box. By clicking this link, available clinical and diagnostic data on this patient will appear beneath the associated genotype. Click **Close Phenotype** and the phenotypic will disappear. Note that in the **Search Genotype** mode detailed phenotypic information is displayed for only one patient at a time.

### 2.3. Phenotype search

This mode allows to directly search for clinical information available on NPC patients submitted to the database. Upon submission, patients are automatically given a specific patient ID (last three digits of PIN) that can be searched for on this page. As some NPC mutations are overrepresented in certain populations, search for ethnic origin may provide

![Search Genotype](image)

![Search Genotype](image)
helpful information. Moreover, Search Phenotype allows queries for patients with a defined clinical course of disease (e.g. by searching for age of onset). A list of frequent clinical symptoms in NPC patients and diagnostic results (e.g. a positive Filipin assay) allows searching for subsets of patients with defined disease presentation. If a clinical symptom is not represented in the list, it may, if submitted, appear when checking Other.

All patients presenting with the queried parameters will appear in the results site.
3. Results display options (View modes)

From the sidebar on Home, two different results view modes of can be accessed: View Summary and View Table. These two view modes provide an overview of either all variants listed in NPC-db or of those variants that fulfil user-defined search criteria. Summary View is of special value for those who seek fast information on a gene or protein sequence variant (e.g. if a certain variant has already been described previously). This view mode can also be accessed by clicking on the shown number of listed gene sequence variants on Home. Table View provides extensive information on each variant in a table format. In addition to genomic location and position in the protein sequence, criteria such as variation type, the probable clinical consequences of the variant or submitter information are displayed in this view.

Details on a certain variant can be accessed by clicking on a highlighted variant in Table view or Summary view. This will lead to a Detail view page that adds associated genotypic and phenotypic data and thus displays the comprehensive information available in NPC-db on a specific variant. Clinical information on Known patients with this variation is listed.
beneath detailed variant sequence information. By clicking on the allelic variant in the field Genotype of a compound-heterozygous patient, Detail view of the respective variant will appear.

4. Submitter Registration

NPC-db is a freely available open-access database. The curators’ expressed aim is to allow users a fast and easy submission of novel data to the database. However, to avoid violation of the database contents, a submitter needs to register before submitting data for the first time. For this, novel users are invited to create a user account in the registration menu which can be accessed from Home by clicking Register now! in the sidebar. For registration the user is asked to provide some obligatory personal information which is indispensable to maintain the quality of a database designed for application in clinical practice. Please use only standard letters and numbers for entries. User information can be edited by the
5. Submission of data

5.1. General remarks

Submission of sequence variation, genotypic and phenotypic data is central to NPC-db. In order to sustain the quality of the database, some rules need to be followed by submitters:

1. Before submission of novel sequence variant entries, please verify your data by comparing it to reference sequences in public databases (e.g. NCBI resources, Ensembl or UCSC Genome Browser; some of these databases can be accessed via the Gene Info page). As adequate sequence information is crucial to NPC-db, please especially cross-check DNA and protein positions for your submitted variant.
2. Check your data for spelling errors (Be aware that if you mix up e.g. the cDNA position when submitting a novel missense mutation, a wrong amino acid will be displayed in the respective protein position!).
3. When entering amino acids, please use the correct one letter code. If you are not sure please use the scroll-down menu in Submit Variation or look up in the literature.
4. Submission of novel variants to NPC-db requires DNA sequence information. Protein sequence information alone is insufficient, as the exchange of one amino acid can be the result of exchanges in more than one nucleotide positions.
5. When submitting clinical information on a specific patient, please make sure that the anonymity of the individual is respected. Upon submission to NPC-db, a patient identification number (PIN) is assigned to every patient. Individuals can be queried for with the last three digits of this PIN via Search Phenotype.

Submission of novel molecular or clinical data to NPC-db can be performed online upon registration. Alternatively, an NPC-db data submission form can be filled manually and be sent it to the curators via email (Heiko.Runz@med.uni-heidelberg.de) or postal services. The NPC-db data submission form can be found at the end of this User Manual. It can also be freely downloaded from the Aims & Scope page.

5.2. Variation submission

For an easy guidance through the online submission procedure of novel sequence variants, Variation Submission is divided into five sub-pages. Pages one to four are for entering data, page five allows to check data for mistakes before submission to the database. Note that once submitted, data will be written directly into the database without delay. As automated error correction is available only for minor entry mistakes, users should check their data very carefully before submission!

5.2.1. Variation submission: page 1

Select the gene in which you want to describe a novel variation (which is not already listed in NPC-db) and check, if the variant is situated in an exon or an intron. In both instances, fill in the number of the respective exon or intron.
5.2.2. Variation submission: page 2
Submission page 2 collects important information on the position of the variation.

First, **DNA information** should be provided, which will most often be available as cDNA information from diagnostic sequencing. If cDNA is checked, a variant will be shown as “c.” followed by its position in the DNA sequence of the gene which needs to be added by the submitter in the field **Position in DNA**. The option **None** is only allowed for intronic variants. As the IVS-nomenclature for intronic sequence variants is outdated, please provide “c.” information also for intron variants if possible [see current HGVS recommendations for the description of DNA sequence variants at http://www.hgvs.org/mutnomen/]. For missense variants, the residue A, T, C or G in the reference sequence and the variant sequence, respectively, should be directly checked. If you want to enter complex variations such as deletions, insertions or duplications, check **Other**. Note that if one of the check boxes is set to **Other**, the field **If other type in here** must be filled with the nomenclature how the variant is supposed to be displayed in the database. For missense mutations this field can be left empty.

Next, the user is asked to provide **protein information**. After entering the number of a respective amino acid residue, amino acids can be selected from a drop down menu. In case the submitted variant will result in a nonsense mutation, select “X”. For complex variants on the protein level fill in a free text in **If other type in here**.

5.2.3. Variation submission: page 3
On submission page 3, sequence **Variation type** can be specified. Please unselect the default setting **I don’t know**, if you can provide details on the type of variation. Second,
Phenotypic consequences (Variation effect) should be provided if available. If the novel variant is disease associated (i.e. it has been identified in a patient considered affected by NPC disease) but has not been reported previously, check Disease associated, novel. If phenotypic consequences of a variant are unknown, select I don’t know.

5.2.4. Variation submission: page 4
On submission page 4, the submitter is asked to provide additional information on the submitted variant if available. E.g., if the variant is a SNP, a respective dbSNP-identifier (e.g. rs004954) can be entered [Note: Please fill in here only numeric values excluding "rs"].

The curators regard it as highly important that the source of the submitted information is displayed in NPC-db. For this, e.g. the submitter’s name or institution followed by “personal communication” or a scientific reference (e.g. by PubMed-ID, PMID) need to be filled into the respective fields as free-text. If the submitter/source is already known to NPC-db from previous submissions, it can be selected from a drop-down menu. All sources contributing to NPC-db are listed on a Reference page that can be accessed from Home by clicking List in the field Number of listed contributors/publications. The field Link can be filled with a www-link to a respective URL (http://...; e.g. on PubMed). If you want to add personal comments...
on the novel variant submitted by you, feel free to enter it into the free text field (Note: Please don’t use semicolons!).

5.2.5. Variation submission: page 5 (Overview)
If you have finished all the previous steps please use this final submission page to check if everything has been entered correctly. In case not, use the edit links and make the respective changes. By clicking Submit your data will be directly submitted to the database. If no error message appears, your variation data has been successfully submitted to NPC-db. Congratulations!
5.3. Submission of Patient Data

A second major feature of NPC-db is the possibility to submit, collect and display information on the genotype and basic clinical features of individual NPC-patients. This is important, not only because it may provide information on the possible course of disease in an individual in which one or more NPC gene sequence variants have been identified. It may also be helpful to link genotype information to the occurrence of specific clinical symptoms.

5.3.1 Genotype Submission

As NPC disease is an autosomal-recessive monogenic disorder, a patient is expected to show at least two disease-causing variants in NPC1 and/or NPC2. The paternal and the maternal disease allele can be either identical for a certain mutation (homozygous), which is frequently observed when parents of a patient are consanguineous. More frequently, however, a patient is compound heterozygous for two different disease-causing alleles.

For submission of genotypic information on a patient, select the up to two allelic disease causing mutations from the drop-down menu. Note that genotype data can only be added for variants that are already listed in NPC-db, but that one variant may appear in combination with many different disease alleles. In case the information should be available, select if the disease causing allele was inherited from the father or the mother in the field Inheritance Status. In the NPC1 gene some missense variants which are found with exceptional frequency are known as synonymous or non-synonymous SNPs (e.g. N931N or I642M). To ease submission, some of these SNPs can be checked in the field Frequent SNPs in NPC1. If the same SNP is checked for allele 1 as well as for allele 2, it will appear in Genotype View as associated homozygous variant. If additional variants or SNPs have been found in a single patient, they can also be entered. For this, first the number of such additional variants must be specified in the field Further variations and a respective number of Associated variation fields will appear. Here the respective information can be entered.
Upon completion of genotype submission, the user is asked to provide clinical details on the respective patient if available (Phenotype submission). Alternatively, another genotype can be entered by clicking Next genotype.

5.3.2. Phenotype submission

The current version of NPC-db does not support the online submission of phenotypic/clinical information without sequence information on a patient. Therefore genotypic data on a patient needs to be submitted prior to clinical information, and the Phenotype submission form can only be entered via Genotype submission. The submitter is first asked for sex and geographic and ethnic origin of the patient. Then, information on disease onset and course, frequent clinical symptoms and results of alternative diagnostic approaches can be checked. Additional information or remarks on specific patients can be entered as free text (please refer to paragraph 5.2.4. on how to do this). For manual data submission, please fill out the NPC-db data submission sheet below and send it to the curators via email or postal services.

Thank you very much for your interest in NPC-db. We hope that you will enjoy using the database and are looking forward to your contributions. Good luck!

Heiko Runz
01/2008
NPC-db data submission form

Thank you very much for submitting your data to NPC-db. Novel data can be submitted to NPC-db either by registering and submitting online at [http://npc.fzk.de](http://npc.fzk.de) or by thoroughly filling this form and send it to the database curators (by email: Heiko.Runz@med.uni-heidelberg.de, by fax: +49-(0)6221-565080 or by postal services). For questions on how to fill out this document, please refer to the NC-db User Manual, which can be downloaded from the Aims & Scope page of NPC-db. In case your information should not appear online until few days upon submission, please directly contact the curators.

1. Submitter information:

| First Name:     | _____________________________________________ |
| Last Name:      | _____________________________________________ |
| Institution:    | _____________________________________________ |
| Phone:          | _____________________________________________ |
| Email:          | _____________________________________________ |

2. Submission of a novel gene sequence variant:

1/4
Select Gene: NPC1 NPC2
Location: Exon Intron
Exon/Intron Number: ___________

2/4
DNA type: cDNA genomic DNA mRNA
Position in DNA: _______________________________
DNA Contig reference: A T G C
DNA missense variation: A T G C
If other, please specify: _______________________________
Position in protein: _______________________________
Amino acid reference: _______________________________
Protein missense variation: _______________________________
If other, please specify: _______________________________
### 3/4

**Variation type:**
- missense
- nonsense
- deletion
- insertion
- frameshift
- SNP, synonymous
- SNP, non-synonymous

**Variation effect:**
- unknown
- disease-associated, novel
- disease-associated, known
- SNP
- SNP-ID (rs…)
- other polymorphism

### 4/4

**Reference article or author:**

**Remarks, Comments, …:**

### 3. Submission of a novel genotype:

**Variation on putative disease causing allele 1:**

<table>
<thead>
<tr>
<th>Inheritance status</th>
<th>from father</th>
<th>from mother</th>
<th>unknown</th>
</tr>
</thead>
</table>

**Variation on putative disease causing allele 2:**

<table>
<thead>
<tr>
<th>Inheritance status</th>
<th>from father</th>
<th>from mother</th>
<th>unknown</th>
</tr>
</thead>
</table>

**Frequent SNPs in NPC1:**
- Y129Y
- H215R
- I642M
- I858V
- N931N

**Have further variants been identified?**

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4. Submission of clinical information on an individual patient:

Identifier (not published online): Initials: ____. Date of birth: / / 

4.1. Sex: male female

4.2. Origin:
Patient resident in: ______________________________________________________
Geographic origin: ______________________________________________________
Ethnic origin: ______________________________________________________

4.3. Clinical course
Age at first clinical symptoms:
no information neonatal (0-1 y) childhood (1-12 y)
adolescent (12-23 y) adult (>23 y)
Age at diagnosis of NPC disease:
no information neonatal (0-1 y) childhood (1-12 y)
adolescent (12-23 y) adult (>23 y)

4.4. Clinical symptoms:
Yes No N/A
Neonatal liver disease
Hepato(spleno)megaly
Seizures
Vertical gaze Palsy
Developmental Delay
Ataxia
Swallowing problems
Pulmonary disease
Mental retardation
Other
If other, please specify: ____________________________________________________

4.5. Diagnostics:
Positive Negative Variant N/A
Filipin assay
Chitotriosidase
Molecular analysis

4.6. Remarks, Comments, …:
________________________________________________________________________
________________________________________________________________________