```
Chicken     -----MRRRLRLLAAALGGCGLLLGSAL-AAGDERLYTAAVMPAL-RVVTPEAAHGLALR
Mouse       MAWRQLRKRALDAAIILGGGGLLFTSYLTATGDDHFYAEYLMPALQRLLDPESAHRLAVR
Macaque     MAWRHLKKRAQDAVVILGGGGLLFASYLMATGDERFYAEHLMPALQGLLDPESAHRLAVR
Chimp       MAWRHLKKRAQDAVIILGGGGLVFASYLMATGDERFYAEHLMPTLQGLLDPESAHRLAVR
Human       MAWRHLKKRAQDAVIILGGGGLLFASYLMATGDERFYAEHLMPTLQGLLDPESAHRLAVR
             :..*     .  **:* **:: * * *:**:.:*:  :**:*  :: **:** **:*
                          └──→ G19E

Chicken     AAALGLLPPAR-PDSPSLEVHVLGLRFRNPLGLAAGFDKHGEAVDGLYRMGFGFVEVGTV
Mouse       VISLGLLPRATFQDSNMLEVRVLGHKFRNPVGIAAGFDKHGEAVDGLYKLGFGFVEVGSV
Macaque     FTSLGLLPRARFQDSDMLEVRVLGHKFRNPVGIAAGFDKHGEAVDGLYKMGFGFVEIGSV
Chimp       FTSLGLLPRARFQDSDMLEVRVLGHKFRNPVGIAAGFDKHGEAVDGLYKMGFGFVEIGSV
Human       FTSLGLLPRARFQDSDMLEVRVLGHKFRNPVGIAAGFDKHGEAVDGLYKMGFGFVEIGSV
             :***** *    **  ***.*** .****:*:************** .:******:*:*

Chicken     TPEPQEGNPKPRVFRLVEDEAVINRYGFNSHGHAAVERRLRARQEAQLKLTTVGMPLGVN
Mouse       TPQPQEGNPRPRVFRLPEDQAVINRYGFNSHGLSAVEHRLRARQQKQTQLTTDGLPLGIN
Macaque     TPKPQEGNPRPRVFRLPEDQAVINRYGFNSHGLSVVEHRLRARQQKQAKLTEDGLPLGVN
Chimp       TPKPQEGNPRPRVFRLPEDQAVINRYGFNSHGLSVVEHRLRARQQKQAKLTEDGLPLGVN
Human       TPKPQEGNPRPRVFRLPEDQAVINRYGFNSHGLSVVEHRLRARQQKQAKLTEDGLPLGVN
             **:****** .****:* **:*************. : ** .******: * :**  *:***:*
                           └──→ R135C      └──→ G152R

Chicken     LGKNKSSTDAAADYVAGVQTLGPLADYLVVNVSSPNTPGLRDLQGKAELRDLLTKVLAER
Mouse       LGKNKTSVDAAADYVEGVRILGPLADYLVVNVSSPNTAGLRSLQGKTELRRLLSKVLQER
Macaque     LGKNKTSVDAAEDYAEGVRVLGPLADYLVVNVSSPNTAGLRSLQGKAELRRLLTKVLQER
Chimp       LGKNKTSVDAAEDYAEGVRVLGPLADYLVVNVSSPNTAGLRSLQGKAELRRLLTKVLQER
Human       LGKNKTSVDAAEDYAEGVRVLGPLADYLVVNVSSPNTAGLRSLQGKAELRRLLTKVLQER
             *****:*.*** **. **|  **|  ************* *** .****:*** **:*** **
             R199C ──→              └──→ L204PfsX8
                               └──→ G202D/A

Chicken     DMLPSERKPAVLVKIAPDLTEQDKQDIAGVVCEVGVDGLIVSNTTTSRPRGLQSTQRLEA
Mouse       DALKGPQKPAVLVKIAPDLTAQDKEDIASVARELGIDGLIITNTTVSRPVGLQGALRSET
Macaque     DGLQGAHRPAVLVKIAPDLTAQDKEDIASVVKELGIDGLIVTNTTVSRPAGLQGALRSET
Chimp       DGLRRVHRPAVLVKIAPDLTSQDKEDIASVVKELGIDGLIVTNTTVSRPAGLQGALRSET
Human       DGLRRVHRPAVLVKIAPDLTSQDKEDIASVVKELGIDGLIVTNTTVSRPAGLQGALRSET
             * *| .:.************ ***:***.*. *:*:****::*|*. *** *** .:  * *:
               └──→ R244W                              └──→ T284I

Chicken     GGLSGKPLRELSTQTVREMYALTQGRV----PIIGVGGVSSGRDALEKIRAGASLVQMYT
Mouse       GGLSGKPLRDLSTQTIREMYALTQGTI----PIIGVGGVSSGQDALEKIQAGASLVQLYT
Macaque     GGLSGKPLRDLSTQTIREMYALTQGKASGIFPIIGVGGVSSGQDALEKIRAGASLVQLYT
Chimp       GGLSGKPLRDLSTQTIREMYALTQGRV----PIIGVGGVSSGQDALEKIRAGASLVQLYT
Human       GGLSGKPLRDLSTQTIREMYALTQGRV----PIIGVGGVSSGQDALEKIRAGASLVQLYT
             *********:*****:*********           ***********.******|*******:**
                                                          └──→ R346W

Chicken     ALVYHGPPVVRTVKRELEELLREQGFKSVMEAVGADHRR
Mouse       ALTFLGPPVVARVKRELEALLKERGFNTVTDAIGVDHRR
Macaque     ALTFWGPPVVGRVKRELEALLKEQGFGRVTDAIGADHRR
Chimp       ALTFWGPPVVGKVKRELEALLKEQGFGRVTDAIGADHRR
Human       ALTFWGPPVVGKVKRELEALLKEQGFGGVTDAIGADHRR
             **.: *****  ****** **.*.**   * :*:*.|*|***
                                              └──→ D392G
```

Supplementary figure 1.

Comparative protein alignment of dihydroorotate dehydrogenase in human, chimp, macaque, mouse, and chicken. The position of each variant identified in patients with Miller syndrome is boxed. Asterisks denote invariable sites and dots indicate conservative substitutions.
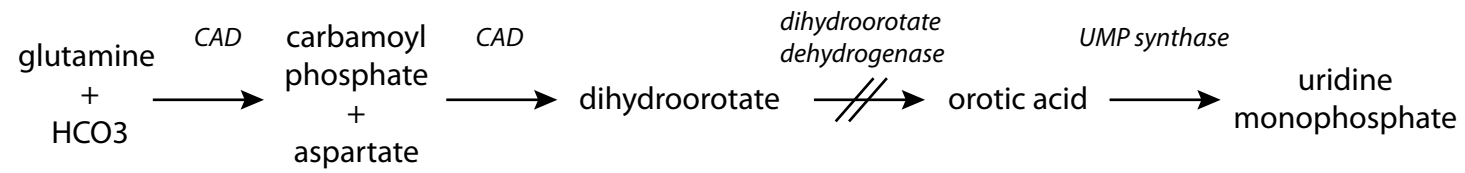
Supplementary Figure 2. Number of candidate genes vs. number of unaffected exomes in filter. The results of adding more unaffected exomes as a filter for novel variants is shown for the recessive model, i.e. requiring at least two novel variants for a gene to qualify as a candidate. For this analysis, either all twelve previously sequenced exomes (Ng et al., 2009) were used (top), or only the eight non-Yoruba exomes from the same study (bottom), and results from all possible combinations of the specified number of exomes are represented. Results of the same filtering with (orange) and without dbSNP129 (blue) are also shown. The single gene identified when using all twelve exomes was DHODH (top); when only non-Yoruba exomes were used, the list also included ESPNL.

Ng, S.B. et al. Targeted capture and massively parallel sequencing of 12 human exomes. *Nature* (2009).

glutamine   *CAD*   carbamoyl   *CAD*   *dihydroorotate*   *UMP synthase*

glutamine + HCO3 →(CAD) carbamoyl phosphate + aspartate →(CAD) dihydroorotate ——//——→(dihydroorotate dehydrogenase) orotic acid ——→(UMP synthase) uridine monophosphate

Supplementary figure 3.

Enzymatic steps controlling *de novo* pyrimidine synthesis. Mutations in *DHODH* are predicted to diminish dihydroorotate dehydrogenase activity.